CSF biomarkers in dementia: Longitudinal aspects and combination with MRI

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CSF biomarkers in dementia: Longitudinal aspects and combination with MRI

Femke H. Bouwman

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dr. N.S.M. Schoonenboom
dr. R.A.I. de Vos

Voor mijn oma
Hendrika H.J. van Workum-Hasselbach

Contents

Chapter I

General introduction and aims of the thesis

Introduction

Alzheimer's disease (AD) is the most common form of dementia. The disease that later became AD was suggested to be a particular form of dementia by the (now famous) German psychiatrist and anatomist Alois Alzheimer, in 1906. He described a woman, Auguste D., 51 years old, who suffered from depression and hallucinations, jealousy towards her husband, amnesia and problems with orientation. In the century that passed after this first case report, the number of AD patients has increased considerably. In the near future the number of AD patients is expected to increase even further especially in developing countries, with great socio-economic consequences.

Diagnosis of AD is made by clinical criteria established by McKhann and colleagues in 1984: patients should have dysfunction of at least two or more areas of cognition (orientation to place and time, memory, language, praxis, attention, visual perception and problem solving skills), with progressive worsening of memory and other cognitive functions, no disturbance of consciousness and onset between ages 40 and 90, most often after age 65.[1] Other systemic disorders and brain diseases that could account for the progressive deficits in memory and cognition should be excluded. Thus, these criteria largely depend on the exclusion of other dementias. The diagnostic accuracy of the clinical criteria is relatively low, with sensitivity of around 80% and specificity of 70%.[2]

In the expectation that new disease modifying or arresting compounds will become available and in view of the expected dramatic increase of AD patients, there is a great need for biological markers to establish an early diagnosis and monitor disease progression and effects of treatment. Therefore, in the past two decades, a fair amount of research has concentrated on the development of these biomarkers and discrimination of patients with cognitive dysfunction, not yet demented, that are at risk for developing AD in the future.

Mild cognitive impairment

The degenerative process of AD probably starts 20-30 years before the clinical onset of AD.[3] During this preclinical period, the neuropathologic AD changes increase, and at a certain threshold the first symptoms, most often impairment of episodic memory, appear.

Great deal of interest has been generated the past two decades concerning the topic of a sort of transitional state between normal aging and dementia, or more

specifically, AD. This condition must be distinguished within the broad range of cognitive functioning that falls outside normal aging. Before coining a new term for this condition (if any) it was recognized as age associated memory impairment, age related cognitive decline, age associated cognitive decline, mild cognitive disorder, mild neurocognitive disorder, cognitively impaired not demented, incipient dementia or isolated memory impairment.[4-9] Mild cognitive impairment (MCI) proposed by Petersen et al, who also suggested criteria for it, is now the most commonly used term for the disorder in individuals who have subjective memory or other cognitive symptoms, objective memory or cognitive impairment and whose activities of daily living are generally normal. The reason to identify subjects with MCI, is to be able to detect AD in an earlier stage of the disease, when therapeutic intervention is most helpful. However, although many patients with MCI have incipient AD, i.e. have early AD pathology and will progress to AD with dementia, others probably have a benign form of MCI as part of the normal aging process. In other patients, cerebrovascular pathology (e.g. infarcts, white-matter lesions), frontotemporal lobar degeneration (FTLD), Lewy body disease (LBD) or a depression may contribute to the symptoms. Thus, progression to clinically diagnosable dementia occurs at a higher rate from MCI than from an unimpaired state, but is clearly not the inevitable clinical outcome at follow-up.[10]

Patients with MCI are of great interest for treatment trials, aiming to alter the progression of MCI to AD. In the MCI stage, however, there is no clinical method to determine which patients will progress to dementia, except for a very long clinical follow-up. Thus, there is a great clinical need for diagnostic instruments to identify incipient AD in MCI cases.

Neuropathology

Microscopically, the neuropathologic changes in AD are characterized by extracellularly located senile plaques and intracellularly located neurofibrillary tangles (figure 1).[11] The main constituent of the extracellular plaques is the protein beta-Amyloid (Aβ).[12] This protein is continuously generated by proteolytic cleavage of its precursor, the amyloid precursor protein (APP)[13]. APP is a single membrane-spanning protein with a large ectodomain and a smaller cytoplasmic tail. APP is metabolized along two pathways. In the non-amyloidogenic pathway, APP is cleaved by a protease referred to as α-secretase resulting in a large N-terminal derivative, α-secretase-cleaved soluble APP (αsAPP), and a smaller C-terminal fragment, C83, which is subsequently cleaved by γ-secretase releasing shorter peptide called p3. In the amyloidogenic pathway, APP is cleaved by a

protease referred to as β-secretase (BACE),[14] resulting in βsAPP and C99. When C99 is subsequently cleaved by γ-secretase, free Aβis released. There are several shorter and longer C-terminal forms of Aβ ($A\beta_{1-37}$, $A\beta_{1-38}$, $A\beta_{1-39}$, $A\beta_{1-40}$, $A\beta_{1-42}$). Of these forms $A\beta_{1-40}$ is the most abundant but $A\beta_{1-42}$ has been found to aggregate more rapidly than Aβ1-40. Futhermore, $A\beta_{1-42}$ is the initial form of Aβ deposited in diffuse plaques, as well as the predominating form of Aβ in senile plaques.[15;16]

The other neuropathological hallmark of AD, intracellularly located neurofibrillary tangles, mainly consist of abnormally hyper-phosphorylated tau. Tau-protein is a microtubule-associated protein located in the neuronal axons. Because of alternative splicing of tau-mRNA, there are six isoforms with molecular masses ranging from 50-65 kDa. The normal function of tau is to promote microtubule assembly and stability by binding to tubulin in the microtubules in the axons.[17] In AD, an abnormally hyperphosphorylated form of tau is the principal component of the paired helical filaments, which make up the neurofibrillary tangles, neuropil threads and senile neuritic plaques.[18] Because of hyperphosphorylation, tau also loses its ability to bind to the microtubules and to stimulate their assembly.[19] Using different techniques, more than 30 phosphorylation sites have been described on tau in the brain.[17]

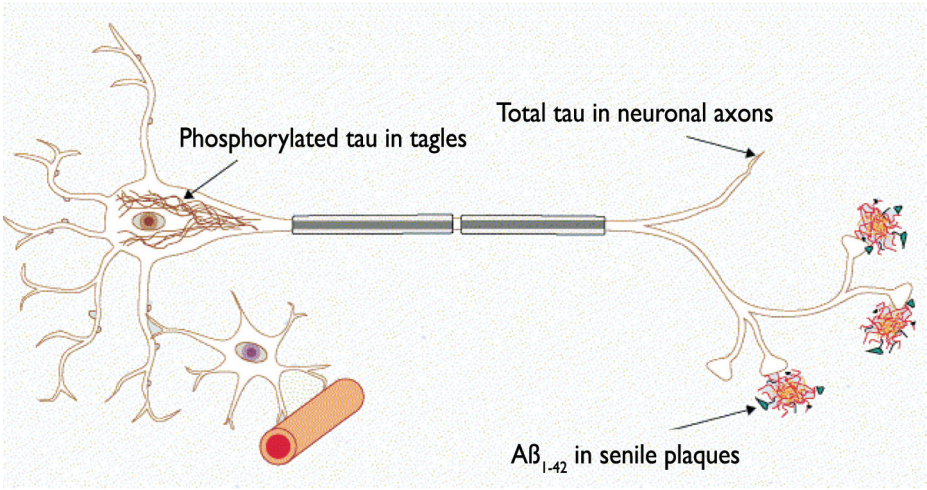

Figure 1

CSF biomarkers

According to the criteria of the Consensus Report of the Working Group on Molecular and Biochemical Markers of AD an ideal biomarker should be 1) able to detect a fundamental feature of Alzheimer's neuropathology, 2) validated in neuropathologically confirmed AD cases, 3) precise (able to detect AD early in its course and distinguish it from other dementias), 4) reliable, 5) non-invasive, 6) simple to perform and 7) inexpensive.[20] Cerebrospinal fluid (CSF) is in direct contact with the extracellular space of the brain, and thus biochemical changes in the brain are thought to be reflected in the CSF. As AD pathology is restricted to the brain, CSF is an obvious source of biomarkers for AD. Since Aβ and (phosphorylated) tau are the main components of the AD neuropathological hallmarks plaques and tangles, research of the past decades has focussed on development of enzyme-linked immunosorbent assay (ELISA) methods for detection of these proteins in CSF.

Beta-Amyloid$_{1-42}$

$A\beta_{1-42}$ has been found to be the most involved in the plaque pathology of AD. Thus, the focus for ELISA methods was specific for $A\beta_{1-42}$.[21] A decrease in CSF $A\beta_{1-42}$ level to about 40-50% of control levels has been found in AD in several studies.[22] The reduced level of $A\beta_{1-42}$ is hypothesized to be caused by deposition of $A\beta_{1-42}$ in plaques, with lower levels diffusing to CSF. This is confirmed by an autopsy study that found strong correlations between low $A\beta_{1-42}$ in ventricular CSF and high number of plaques in the neocortex and hippocampus [23]. In addition, a recently published PIB-PET study indicated that amyloid load is inversely related to CSF $A\beta_{1-42}$ level in humans.[24] Other less current hypotheses, for the reduced CSF $A\beta_{1-42}$ level in AD patients are: 1) decreased production of $A\beta_{1-42}$ by less (active) neurons, as a result of neurodegeneration or 2) altered binding to $A\beta_{1-42}$ specific proteins (e.g. ApoE), resulting in masking of the epitope, to which the antibodies of the assays are directed.

Total tau

During the last 20 years ELISA methods have been developed based on monoclonal antibodies that detect all isoforms of tau, independent of phosphorylation state of tau. An increase of CSF total tau in AD has consistently been found in numerous studies, with a mean of three times higher level in AD than in controls.[22] In acute conditions such as stroke or head trauma, there is a marked transient increase in CSF total tau.[25] Furthermore, the level of increase in CSF total tau is highest in disorders with the most intense neuronal degeneration, such as Creutzfeldt-Jakob disease (CJD).[26;27] In AD a

moderate to marked increase of CSF total tau is found which is in line with less intense degeneration. Normal levels are found in patients with depression, with no or limited degeneration. Thus the CSF level of total tau probably reflects the intensity of neuronal damage and degeneration in general.

Phosphorylated tau

For several different phosphorylated derivatives of tau (amongst others threonine 181, threonine 231, serine 199) ELISA methods have been developed. A marked increased level of phosphorylated tau (ptau) in CSF in AD patients has been found for all these different ELISA methods. In contrast to total tau, there is no change in CSF ptau after acute stroke and normal levels are found in CJD, despite a marked increase in total tau.[25,26] This indirect evidence suggests that CSF ptau is not simply a marker for neuronal damage, like CSF total tau, but that it specifically reflects the phosphorylation state of tau. Clinical studies show that CSF levels of $A\beta_{1-42}$ and (p)tau differentiate AD from controls, Parkinson's disease (PD) or FTLD with reasonable accuracy.[29-31] Ptau-181 has been suggested to discriminate AD from dementia with Lewy bodies (DLB) and PD from Parkinson's disease with dementia (PDD), although overlap occurs between groups.[32;33]

Longitudinal CSF biomarker measurements

Longitudinal changes of CSF biomarkers, such as $A\beta_{1-42}$, are of potential use to study the course of the disease and effects of treatment in dementia. The variability of $A\beta_{1-42}$ levels, effects of CSF processing and assay variability, however, complicate studies of longitudinal $A\beta_{1-42}$ changes.[34;35] Very few studies have described longitudinal changes of $A\beta_{1-42}$, total tau and ptau levels in CSF, mostly in small samples of AD patients.[36-43]. The results of these studies are conflicting, the majority showing no significant changes of these CSF biomarkers, some showing a decrease and others showing an increase over time. Despite these inconclusive findings, clinical trials using CSF markers as outcome parameters have been reported and are being carried out.[39-45] Few studies with small patient samples, described longitudinal changes of CSF biomarkers in MCI patients.[37;38]

MRI

In AD, the earliest neuropathological changes occur in the medial temporal lobe, including the hippocampus and parahippocampal gyrus. The medial temporal lobe plays an important role in the storage of new information, explaining why memory dysfunction is an early symptom of AD. Atrophy of the medial temporal lobe can be detected by Magnetic Resonance Imaging (MRI), and is a sensitive marker for AD also in an early stage.[46] Previous studies have shown that the volume of the medial temporal lobe is a marker for future dementia in patients with MCI.[47] Also, whole-brain atrophy rate, measured from serial MRI, correlates well with disease and clinical progression in patients with MCI and AD.[48;49] However, volumetric assessment is difficult to apply in routine clinical practice because of the time-consuming ROI analysis and not widely availability of the volume measurement techniques. The assessment of medial temporal lobe atrophy (MTA) using a standardized visual rating scale [50] is a quick and easy measurement with a predictive accuracy of AD in MCI patients comparable to volumetric assessment of the hippocampus.[51;52]

Combination of CSF biomarkers with MRI

Both MTA, whole-brain atrophy rate and CSF biomarkers have been shown to be able to predict AD in patients with MCI.[51-55] Neither CSF nor MRI, however, achieves 100% specificity and sensitivity to predict AD in MCI patients. Few studies combined these markers for prediction of AD in MCI patients. Very recently, researchers defined new research criteria for AD that allow diagnosis when symptoms first appear, before full-blown dementia. Thereby the MCI construct would be eliminated, thus supporting earlier intervention at the prodromal stage. Dubois et al. propose to make use of CSF or MRI or FDG-PET scan combined with a memory delayed recall test.[56]

Aims of the thesis

The objective of this study was to explore longitudinal changes of CSF biomarkers and post mortem levels of these CSF biomarkers. In addition the combined value of CSF and MRI was compared, for discriminating (incipient) AD in memory clinic patients.

In chapter 2, we describe changes of CSF $A\beta_{1-42}$ level with time in individual patients and assessed the influence of assay variation on storage of specimen on CSF $A\beta_{1-42}$ level. Subsequently the natural course of CSF $A\beta_{1-42}$, total tau and ptau-181 levels over time is described in AD and MCI patients as well as in patients with subjective complaints only. In addition stable and progressive MCI patients are compared for their change of biomarker level.

In chapter 3, CSF levels of $A\beta_{1-42}$, total tau and ptau-181 are compared between AD and controls according to age with the ultimate goal to gain further insight in possible differences of AD pathology in young and old patients.

In chapter 4, levels of $A\beta_{1-42}$, total tau and ptau-181 are studied in post mortem CSF of controls and patients with AD and LBD.

In chapter 5, we first set out to study CSF biomarkers and MTA in their ability to predict dementia in MCI patients and assessed the predictive value of the combination of these biomarkers. Subsequently we assessed associations between baseline and longitudinal CSF biomarker levels and whole-brain atrophy rate in MCI and AD patients, and the ability of these markers to predict disease progression. Finally, both CSF and MRI are combined with memory score to assess the value of the newly proposed research criteria for AD.[56]

In chapter 6, the main findings of the thesis are summarized followed by a discussion with reference to the literature, and implications and recommendations for future research.

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Chapter 2

Longitudinal measurements of CSF biomarkers

Feasibility and usefulness of longitudinal beta-Amyloid$_{1-42}$ levels in cerebrospinal fluid of patients with various cognitive and neurological disorders

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Abstract

Objective: Longitudinal changes in CSF biomarkers, that are of potential use to study the course of the disease and effects of treatment, have been studied scarcely. We evaluated changes in CSF beta-Amyloid$_{1-42}$ (Aβ_{1-42}) levels with time and assessed the influence of variability of assays and storage of specimen on the results.

Methods: 114 patients (54% male, age 67±9 years) with various cognitive and neurological disorders were included. All patients underwent two spinal taps (mean follow-up time 21 months). Aβ_{1-42} was measured with a sandwich ELISA (Innotest β-Amyloid$_{1-42}$; Innogenetics, Ghent, Belgium). Baseline samples were assayed twice: once shortly after the first spinal tap and once, in a separately stored aliquot, concomitant with the follow-up sample. Variances of coefficients of variation were calculated.

Results: Mean Aβ_{1-42}-level at baseline was 485±242 pg/ml. Mean Aβ_{1-42}-level at follow up was 520±249 pg/ml (p=0.00). Repeated mean baseline Aβ_{1-42}-level was 477±232 pg/ml. Variance of the baseline and follow up CSF-sample pairs run on the same assay was 10% and 18% for pairs run on separate assays. Variance of repeated Aβ_{1-42} assessment in baseline CSF-samples was 14%. Storage time was found not to be associated with differences in Aβ_{1-42}-level.

Conclusion: In case of repeated spinal taps, determination of Aβ_{1-42}-levels should be performed in the same assay. However, the measuring error of repeated Aβ_{1-42} assessment exceeds the biological changes over time. This may imply, that with the methodological limitations of the present ELISA, repeated Aβ_{1-42}-assessment is not feasible in a clinical setting.

Introduction

Measuring protein levels in cerebrospinal fluid (CSF) has gained wide acceptance for the differential and early diagnosis of dementia.[1-3] With upcoming disease arresting compounds, there is a great need for biological markers to monitor disease progression and effects of treatment. Longitudinal changes of CSF biomarkers, such as β-Amyloid$_{1-42}$ (Aβ_{1-42}), are of potential use to study the course of the disease and effects of treatment in dementia.

The variability of Aβ_{1-42}-levels and effects of CSF processing, however, complicates studies of longitudinal Aβ_{1-42} changes. A meta-analysis demonstrated considerable variability in absolute concentrations of Aβ_{1-42} among centres, even when using the same commercial assay.[4] And a recent study demonstrated the influence of repeated freeze/thaw cycles and storage temperature on the concentration of Aβ_{1-42}.[5;6] Especially when studying longitudinal changes of CSF biomarkers to evaluate disease progression or treatment effects, sample stability and assay variability are important issues.

Only few studies described longitudinal changes of Aβ_{1-42} level in CSF and little is known about the methodological issues associated with it.[7-12] In this study we evaluated changes in CSF Aβ_{1-42}-level with time and assessed the influence of assay variation and storage of specimen on the results in patients with various cognitive and neurological disorders.

Methods

Participants

114 patients were recruited at the Alzheimer Centre of the VU Medical Centre (VUMC) between November 2000 and October 2004. All patients underwent two spinal taps. Mean follow-up time, defined as the time between the first and second lumbar puncture (LP), was 21 months (SD 9). The ethical review board of the VUMC approved the study and all subjects gave written informed consent.

CSF analysis

CSF was obtained by LP between the L3/L4 or L4/L5 intervertebral space, using a 25-gauge needle, and collected in 12-mL polypropylene tubes. Within two hours, CSF samples were centrifuged at 2100*g* for 10 minutes at 4°C. A small amount of CSF was used for routine analysis, including total cells (leucocytes and erythrocytes), total protein and glucose. CSF was aliquoted in polypropylene tubes of 0.5 or 1 ml, and stored at -80°C until further analysis.

$A\beta_{1-42}$ was measured with a sandwich ELISA (Innotest β-Amyloid$_{1-42}$; Innogenetics, Ghent, Belgium).[13] Baseline samples were assayed twice, i.e. once shortly after the first spinal tap (A1) and once, in a separately stored aliquot (A2), concomitant with the follow-up sample (B) (please note that storage time of the baseline sample equals follow-up time).

The mean intra-assay coefficient of variation (CV, averaged (SD/mean)*100%) was 2.8% in duplicate samples of four different assays. The inter-assay CV of 6.9%-12.6% was determined by calculating the CV for four different quality control samples: two with a mean $A\beta_{1-42}$ of 603 pg/ml and 612 pg/ml run 11 across assays between January 2004 and December 2004 and two other samples with a mean $A\beta_{1-42}$ of 448 pg/ml and 570 pg/ml run 15 across assays between December 2004 and December 2005.

Statistical analysis

For statistical analysis, SPSS version 12.0 (Chicago, IL) was used. Paired samples Student's T-test was used to evaluate changes in $A\beta_{1-42}$-levels within subjects. The CV for baseline and follow-up sample pairs was calculated. CV's were visualized in Bland Altman plots and compared using Pitman's test.[14] To calculate correlation between storage time and changes in CSF $A\beta_{1-42}$-levels Pearson correlation coefficient was used. Significance was set at $p<0.05$.

Results

Table 1 summarizes the demographic characteristics of the study population. Mean time between first and second spinal tap was 21±9 months. Mean $A\beta_{1-42}$-level at baseline was 485±242 pg/ml (A1). Mean $A\beta_{1-42}$-level at follow up was 520±249 pg/ml (B) (table 1). Mean difference between baseline and follow-up $A\beta_{1-42}$-level was 35±154 pg/ml ($p<0.01$) for CSF-samples run in different assays (A1-B) and 43±82 pg/ml ($p<0.01$) for CSF samples run in the same assay (A2-B). Repeated mean baseline $A\beta_{1-42}$-level was 477±232 pg/ml (A2). Mean difference between first and second assessment of baseline $A\beta_{1-42}$-level in different assays was 8±123 pg/ml ($p=0.5$) (A1-A2).

In figure 1 the variances of the CV's within subjects are illustrated in Bland Altman plots. The CV of baseline and follow up CSF-sample pairs ran on the same assay (A2-B) was 10% (fig 1A). By contrast a CV of 18% (fig 1B) was found within CSF-sample pairs run on separate assays (A1-B). The CV of repeated $A\beta_{1-42}$ assessment in baseline CSF-samples (A1-A2) was 14% (fig 1C). Pitman's test [14] demonstrated a significant difference between these variances ($p<0.001$).

Storage time was found not to be associated with differences in baseline $A\beta_{1-42}$-level (r=0.15, p=0.12).

Table 1. *Demographic characteristics of subjects (n=114) with baseline and follow up CSF β-Amyloid$_{1-42}$-levels*

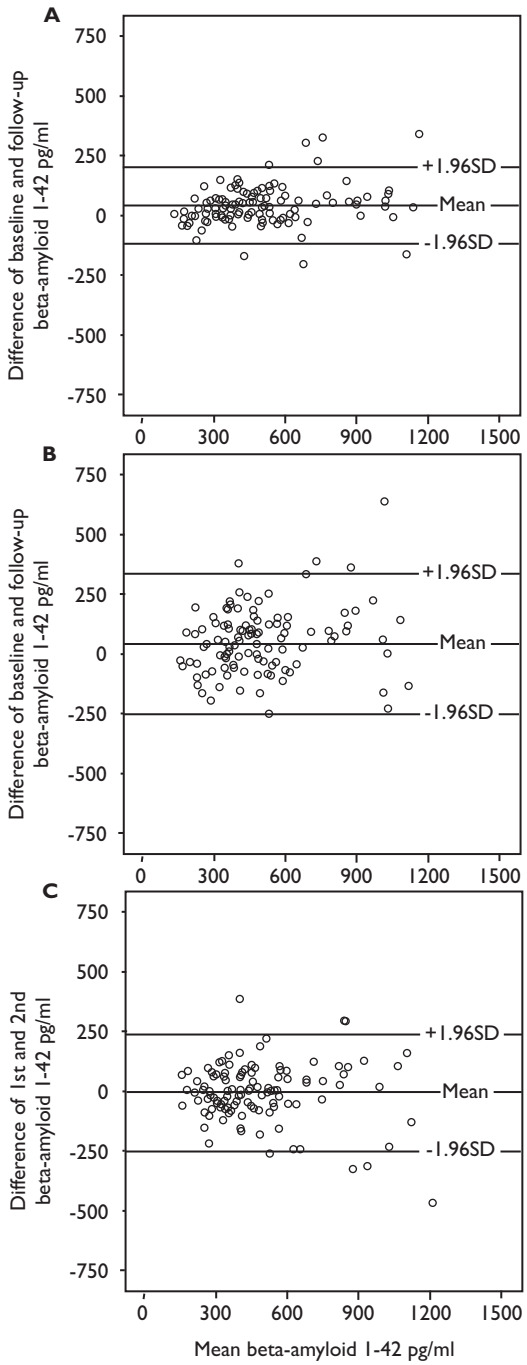
Men/Women	61/53
Age in years	67 (9)
MMSE	24 (5)
Follow up time in months	21 (9)
Diagnosis: AD, MCI, SMC, OND (%)	44, 33, 15, 8
Baseline β-Amyloid$_{1-42}$ (A1)	485 (242)
Follow up β-Amyloid$_{1-42}$ (B)	520 (249)
Baseline β-Amyloid$_{1-42}$ repeated (A2)	477 (232)

MMSE=mini mental state examination, AD=Alzheimer's disease, MCI=Mild Cognitive Impairment, SMC=subjective memory complaints, OND=other neurological diseases. Data are represented as mean (SD), unless indicated otherwise. β-Amyloid$_{1-42}$-levels are in pg/ml.

Discussion

The main finding of this study is the higher variability of baseline and follow-up $A\beta_{1-42}$-levels when assessed in different assays compared to assessment in the same assay. This suggests that the measuring error exceeds the biological changes over time. Therefore, in case of repeated spinal taps, determination of $A\beta_{1-42}$-levels should be performed in the same assay.

The variability of $A\beta_{1-42}$-levels may be caused by methodological limitations of the $A\beta_{1-42}$ ELISA assay. Another possible cause of variability of baseline $A\beta_{1-42}$-levels in our study is higher variability at higher $A\beta_{1-42}$-levels compared to lower levels as suggested in fig 1. There was no essential change of variability, however, after exclusion of the 12 highest mean $A\beta_{1-42}$-levels from analysis (results not shown). In addition, no change in variability was found when we restricted the analysis to CSF-samples determined after 2003 (results not shown), so there was no significant influence of growing experience with the ELISA-method in our laboratory over the years. Also, difference in follow-up time and thus storage time of CSF-samples might have caused variability, since different $A\beta_{1-42}$-forms may degrade after several years of storage at -80°C. We did not find an association, however, between follow-up time (i.e. storage time) and changes in repeated baseline $A\beta_{1-42}$-level. This confirms earlier data on effects of processing and storage conditions on $A\beta_{1-42}$-levels.[5;6]



1A. *Baseline and follow-up CSF-sample in the same assay. Variance 10%.*

1B. *Baseline and follow-up CSF-sample in different assays. Variance 18%.*

1C. *Baseline CSF-sample in different assays. Variance 14%.*

Figure 1. *Bland-Altman plots of $A\beta_{1-42}$ concentrations in baseline and follow-up samples*

Since ELISA's for measuring $A\beta_{1-42}$-levels are available now more than ten years, it is striking that there are only few studies of changes of $A\beta_{1-42}$-levels over time. One study showed that $A\beta_{1-42}$-levels decrease over time, while the other studies showed no significant changes of $A\beta_{1-42}$-levels over time.[7-10] Remarkable in all these longitudinal studies is that only a few mention intra and inter-assay variability and no study explicitly reports that baseline and follow up CSF-samples were assessed in the same assay. All above mentioned studies report wide ranges and/or standard deviations of $A\beta_{1-42}$-levels, corresponding with our finding of large variances. In particular, in our study the variance of the difference of baseline and follow-up CSF-samples in the same assay is smaller than the variance of the difference of repeated $A\beta_{1-42}$-assessment of the baseline CSF-sample in different assays. This suggests that, even with acceptable within and between assay variation as judged from the results of the quality control pools, the biological change over time within subjects is smaller than the measuring error. The ultimate implication of this finding may be, that with the methodological limitations of the present ELISA, repeated $A\beta_{1-42}$ determination is not useful in a clinical setting. In addition, repeated $A\beta_{1-42}$-assessment in clinical trials as biomarker of progression in AD may be premature at this stage.

The aforementioned studies included patients with cognitive neurodegenerative disorders such as AD, DLB and mild cognitive impairment.[7-11] In our study, a variety of patients was used with various cognitive and neurological disorders. This enabled us to analyse variability in a wide range of $A\beta_{1-42}$-levels in a large cohort of patients. The biological significance of repeated spinal taps in individual patients remains to be established.

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Longitudinal changes of CSF biomarkers in memory clinic patients

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Abstract

Objective: In Alzheimer's disease (AD), longitudinal changes of beta-Amyloid$_{1-42}$ (Aβ_{1-42}), tau and phosphorylated tau at threonine-181 (ptau-181) in cerebrospinal fluid (CSF) have been reported in small studies only. We evaluated the natural course of CSF biomarkers in patients with AD, subjective complaints and mild cognitive impairment (MCI).

Methods: 105 patients (50 AD, 38 MCI, 17 subjective complaints) underwent two lumbar punctures, with mean interval of 21±9 months. CSF levels of Aβ_{1-42}, tau and ptau-181 were measured.

Results: CSF Aβ_{1-42} and tau-levels showed main effects for both diagnosis and time (all $p<0.05$) with an average increase of 47±72 pg/ml and 49±143 pg/ml. The interaction terms were not significant, which implies a similar time effect for all diagnostic groups. CSF ptau-181 levels showed a main effect for diagnosis ($p=0.01$), but not for time ($p=0.27$, increase of 1.0±12 pg/ml).

Conclusion: CSF Aβ_{1-42} and tau, but not ptau-181 levels increased over time in this memory clinic patient cohort with comparable change in all diagnostic groups. The cross-sectional difference between diagnostic groups, however, exceeded by far the longitudinal changes within individuals, suggesting that these biomarkers are not sensitive as markers of disease progression.

Introduction

Dementia is a rapidly growing socioeconomic and medical problem.[1] Alzheimer's disease (AD) is the most common cause of dementia.

The major histopathological hallmarks of AD are senile plaques, containing beta-Amyloid$_{1-42}$ (Aβ_{1-42}) and neurofibrillary tangles with microtubuli-associated tau protein. These pathological changes are present before the onset of clinical dementia.[2] Decreased Aβ_{1-42} levels in cerebrospinal fluid (CSF) and increased tau and phosphorylated tau (ptau) levels can differentiate patients with AD from control subjects or patients with other neurological conditions with relatively high accuracy.[3-5] Moreover, these CSF changes can already be detected in patients with mild cognitive impairment (MCI) who later develop AD.[6,7]

Although of great importance to gain insight in the natural course of these biomarkers within individuals, only small studies described longitudinal changes of Aβ_{1-42}, tau and ptau levels in CSF, mostly in AD patients.[8-15] The results of these studies are contradictory, the majority showing no significant changes of these CSF biomarkers,[8,11,12,14] some showing a decrease[15] and others showing an increase[10,13] over time. Despite these inconclusive findings, clinical trials using CSF markers as outcome parameters have been reported and are being carried out.[10,16,17] Few studies with small patient samples, described longitudinal changes of CSF biomarkers in MCI patients.[11,12]

In this study we evaluated the natural course of CSF biomarker levels over time in AD and MCI patients as well as in patients with subjective complaints only. In addition, stable and progressive MCI patients were compared for their change of biomarker level.

Methods

Study population

105 patients (50 with AD, 38 with MCI and 17 with subjective complaints) who underwent two lumbar punctures were recruited at the Alzheimer Center of the VU Medical Center (VUMC) between November 2000 and November 2005. At baseline patients underwent a standardized clinical assessment, including medical history, physical and neurological examination including Mini Mental State Examination (MMSE),[18] laboratory tests, psychometric evaluation, EEG and brain MRI. Follow-up investigation was performed, during which patients were asked to undergo a second lumbar puncture.

At least one year was required between first and second lumbar puncture. For one AD patient baseline MMSE was not available and for 18 subjects follow-up MMSE was not available (11 AD, 4 MCI, 4 subjective complaints). Mean follow-up time, defined as the time between first and second LP, was 21±9 months. Baseline and follow-up diagnoses were made by a multidisciplinary team of neurologists, neuropsychologists, a neurophysiologist, a psychiatrist, a geriatrician and a radiologist. Criteria of Petersen et al. were used for MCI[19,20] and NINCDS-ADRDA criteria for AD.[21] When all clinical investigations were normal, patients were considered to have subjective complaints. The team involved in the diagnostic work-up was not aware of the results of the CSF analyses. The study was approved by the ethical review board of the VUMC and all subjects gave written informed consent.

CSF analysis

CSF was obtained by LP between the L3/L4 or L4/L5 intervertebral space, using a 25-gauge needle, and collected in 12-mL polypropylene tubes. Within two hours, CSF samples were centrifuged at 2100*g* for 10 minutes at 4°C. A small amount of CSF was used for routine analysis, including total cells (leucocytes and erythrocytes), total protein and glucose. CSF was aliquoted in polypropylene tubes of 0.5 or 1 ml, and stored at −80°C until further analysis. CSF $A\beta_{1-42}$, tau and tau phosphorylated at threonine-181 (ptau-181) were measured by commercially available sandwich ELISAs (Innotest beta-Amyloid$_{1-42}$,[22] Innotest hTau-Ag,[23] and Innotest Phosphotau $_{(181P)}$,[24] Innogenetics, Ghent, Belgium). The team involved in the CSF analysis was not aware of the clinical diagnoses. For two patients ptau-181 levels were not available. The intra-assay coefficient of variation (CV) for $A\beta_{1-42}$ was 2.8%, 3.7% for tau and 1.6% for ptau-181. To circumvent inter-assay variability, baseline and follow-up samples were run in the same assay at the time of the second spinal tap.[25]

Statistical analysis

For statistical analysis, SPSS version 12.0 (Chicago, IL) was used. One way Analysis of Variance (ANOVA) with post hoc Bonferroni tests was used to compare continuous variables between the diagnostic groups. Frequency distributions for sex were compared with chi-squared tests. Absolute change over time in CSF markers was assessed using ANOVA's for repeated measures, with diagnosis as between-subjects variable, time as within-subjects variable and CSF $A\beta_{1-42}$, tau and ptau-181 levels as dependent variables.

To evaluate the biological significance of repeated measurements of biomarkers, the critical difference, expressed as a percentage, was calculated with the formula:

$CD = Z \times \sqrt{2} \times \sqrt{(CV_A^2 + CV_I^2)}$, where CV_A is the coefficient of variation due to analytical imprecision, CV_I the average inherent within-subject biological variation and Z the Z-score (=1.96 at 0.95 confidence level).[26] We estimated the combined analytical and within subject random biological variation ($=\sqrt{(CV_A^2 + CV_I^2)}$) of CSF $A\beta_{1-42}$, tau and ptau-181 as 10%, 11% and 8% based on the coefficient of variation we found in an earlier study assessing baseline and follow-up samples of 114 subjects in the same assay.[25]

Results

Demographic characteristics are presented in table 1. No differences were found for sex, age, and follow-up time between the patient groups. MMSE scores of patients with MCI and AD declined over time, while scores of patients with subjective complaints remained stable.

Table 1. *Demographic characteristics of diagnostic groups*

	SC (n=17)	MCI (n=38)	AD (n=50)
Men/Women	11/6	20/18	24/26
Mean age	65 (11)	69 (8)	66 (8)
Follow-up time (months)	25 (14)	20 (8)	21 (8)
MMSE at baseline#a	28 (2)	26 (3)	22 (5)
MMSE at follow-up#b	28 (1)	24 (3)*	18 (5)*

Data are presented as mean (standard deviation) unless indicated otherwise.
SC=subjective complaints, MCI=mild cognitive impairment, AD=Alzheimer's disease. MMSE=mini mental state exam.
# Baseline MMSE was missing for 1 AD patient, follow-up MMSE was missing in 18 subjects. [a] AD<MCI=SC ($p<0.05$). [b] AD < MCI < SC ($p<0.05$). * decrease over time ($p<0.05$) .

Mean baseline and follow-up levels of CSF $A\beta_{1-42}$, tau and ptau-181 are presented in table 2.

ANOVA for repeated measures revealed main effects for $A\beta_{1-42}$ and tau for both diagnosis and time (see table 2 for statistical details). CSF $A\beta_{1-42}$ and tau levels showed an increase of 47±72 pg/ml and 49±143 pg/ml (see also figure 1). The interaction term was not significant, which implies a similar time effect for all diagnostic groups (see also figure 2). CSF ptau-181 levels showed a main effect for diagnosis but not for time (increase of 1.0±12 pg/ml). The interaction term was not significant.

As for absolute changes, no difference in percentage changes of biomarkers over time was found between the patient groups. The critical differences, of $A\beta_{1-42}$ tau and

ptau-181 were estimated to be 28%, 30% and 22%. As expected most subjects did not exceed the critical difference. Of subjects who did exceed the critical difference ($A\beta_{1-42}$ 15, tau 11, ptau 9), most increased over time, and some decreased ($A\beta_{1-42}$ 2/15, tau 1/11, ptau 3/9). The number of patients exceeding the critical difference was equally distributed among the diagnostic groups for all three biomarkers (all p>0.34).

Table 2. *Mean baseline and follow-up levels of $A\beta_{1-42}$, tau and ptau-181 in diagnostic groups*

		SC (n=17)	MCI (n=38)	AD (n=50)
$A\beta_{1-42}$	Baseline	691 (215)	486 (214)	380 (166)
	Follow-up	735 (276)	531 (235)	429 (176)
	% change	6 (18)	10 (15)	14 (19)
Tau	Baseline	365 (199)	650 (696)	731 (363)
	Follow-up	396 (216)	694 (745)	791 (419)
	% change	10 (15)	7 (18)	11 (30)
Ptau-181#	Baseline	53 (22)	73 (37)	84 (34)
	Follow-up	56 (22)	74 (39)	85 (35)
	% change	6 (11)	2 (14)	3 (23)

Data are presented as mean (standard deviation). $A\beta_{1-42}$ =beta-Amyloid$_{1-42}$, ptau-181=tau phosphorylated at threonine-181, SC=subjective complaints, MCI=mild cognitive impairment, AD=Alzheimer's disease. # Ptau-181 was missing for 2 patients.

ANOVA for repeated measures revealed main effects for $A\beta_{1-42}$ and tau for both diagnosis ($A\beta_{1-42}$: $F(2,102)=15$, $p<0.01$; tau: $F(2,102)=3.5$, $p<0.05$) and time ($A\beta_{1-42}$: $F(1,102)=34$, $p<0.01$; tau: $F(1,102)=8.2$, $p<0.01$). There was no interaction between time and diagnosis ($A\beta_{1-42}$: $F(2,102)=0.45$, $p=0.96$; tau $F(2,102)=0.31$, $p=0.73$). Ptau-181 showed a main effect of diagnosis ($F(2,100)=4.9$, $p=0.01$), but not for time ($F(1,100)=1.2$, $p=0.27$, increase of 1.0±12 pg/ml). Again, the interaction term was not significant.

ANOVA of % changes over time showed no differences between groups ($A\beta_{1-42}$: $F(2,102)= 1.5$, $p=0.22$; tau: $F(2,102)=0.28$, $p=0.76$; ptau: $F(2,100)=0.31$, $p=0.74$). T-test of % changes showed increases for $A\beta_{1-42}$ and tau (11% and 9 %, $p<0.01$) but not for ptau ($p=0.13$).

In an additional analysis using ANOVA for repeated measures, stable MCI patients were compared to progressive MCI patients. At follow-up, 13 of 38 MCI patients remained stable and 25 had progressed to dementia: 21 to AD, two to vascular dementia,[27] one to frontotemporal lobar degeneration,[28] and one to dementia with Lewy Bodies.[29] Mean baseline and follow-up levels of CSF $A\beta_{1-42}$, tau and ptau-181 are presented in table 3. $A\beta_{1-42}$ showed main effects for both MCI-group and time. For tau there was a main effect of time, while the effect of MCI-group was borderline significant. For ptau-181 there was a main effect of MCI-group but not of time. The interaction terms were not significant.

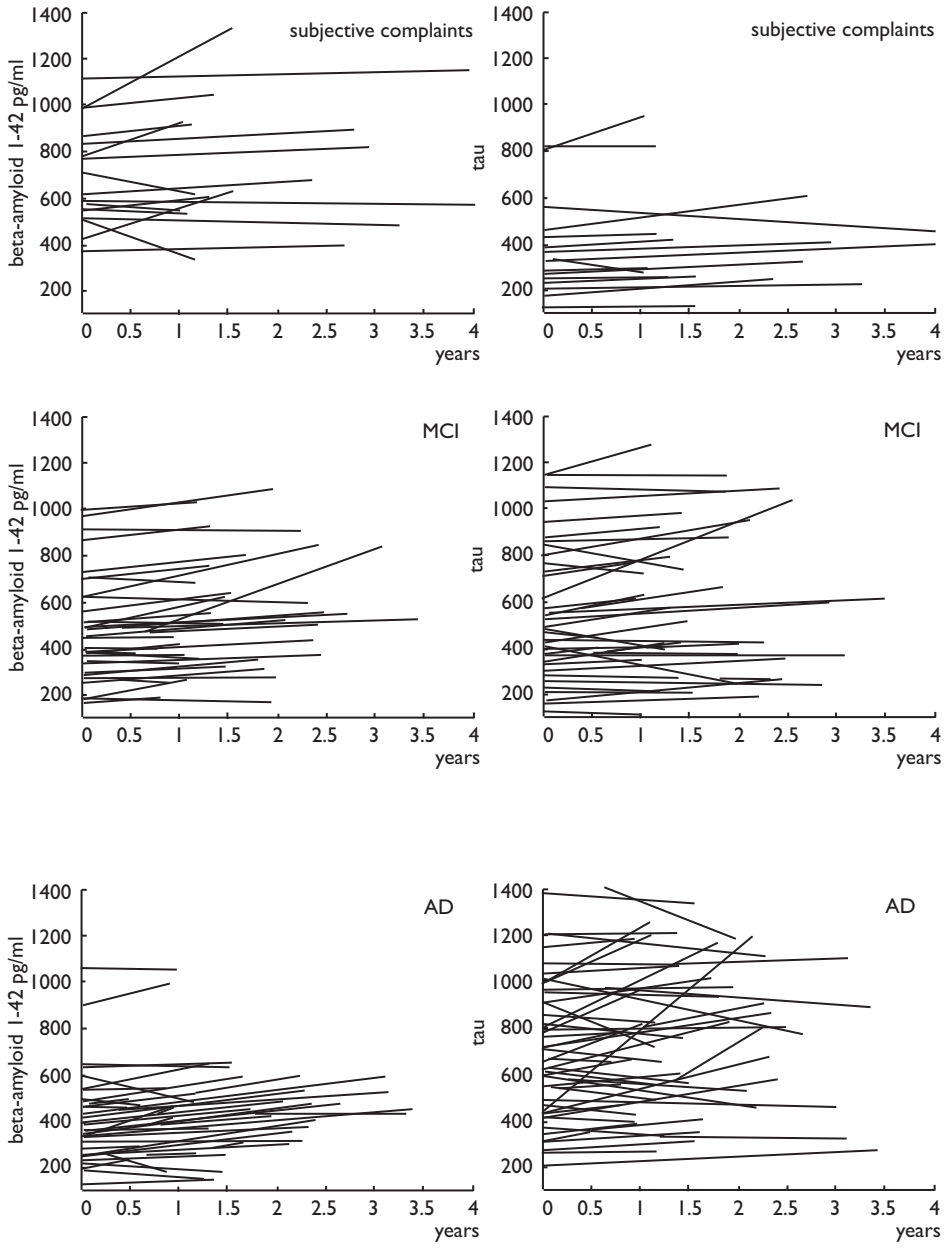

Figure 1. *Rate of change over time in Aβ $_{1-42}$, tau and ptau-181 levels for each patient*

MCI=mild cognitive impairment, AD=Alzheimer's disease. Rate of change over time in biomarker levels is presented for each individual patient; each panel represents a different diagnostic group. Time=0 is set to the date of the first lumbar puncture.

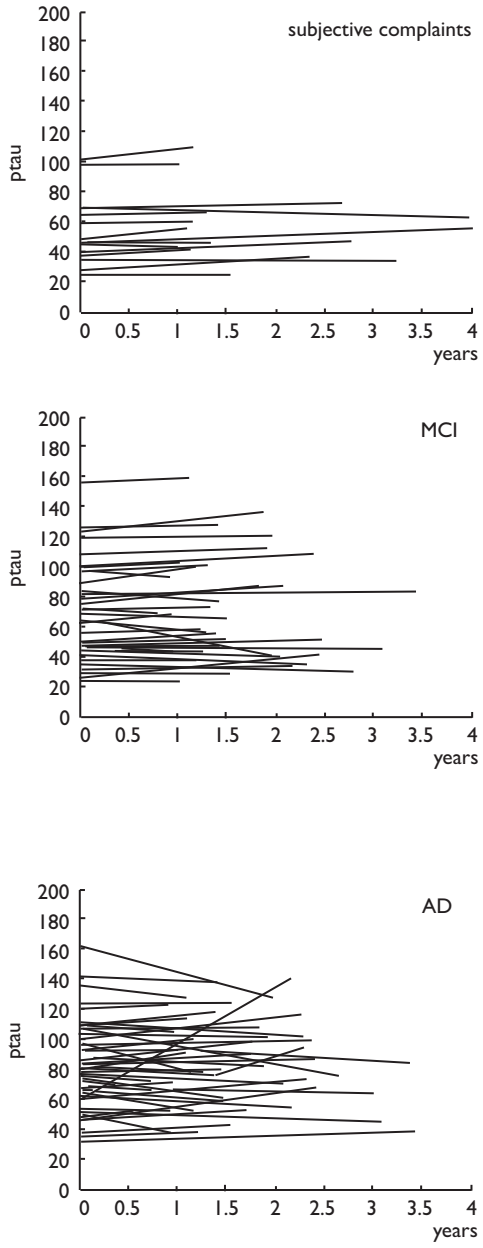

Figure 1. *(continued)*

Table 3. *Mean baseline and follow-up levels of $A\beta_{1-42}$, tau and ptau-181in stable and progressive MCI patients*

		MCI stable (n=13)	MCI progressive (n=25)
$A\beta_{1-42}$	Baseline	611 (232)	421 (176)
	Follow-up	677 (249)	456 (192)
	% change	12 (12)	9 (16)
Tau	Baseline	357 (226)	803 (806)
	Follow-up	376 (246)	859 (864)
	% change	6 (22)	8 (16)
Ptau-181#	Baseline	55 (27)	83 (39)
	Follow-up	54 (28)	85 (41)
	% change	1 (21)	2 (8)

Data are presented as mean (standard deviation). $A\beta_{1-42}$ =beta-Amyloid$_{1-42}$, ptau-181=tau phosphorylated at threonine-181, MCI=mild cognitive impairment. # Ptau-181 was missing for 2 patients.
ANOVA for repeated measures revealed main effects for $A\beta_{1-42}$ of both MCI group ($F(1,36)=8.9$, $p<0.05$) and time ($F(1,36)=13$, $p<0.01$), tau showed main effect of time ($F(1,36)=4.9$, $p<0.05$) and borderline significant effect of MCI group ($F(1,36)=3.8$, $p=0.06$), ptau-181 showed main effect of MCI group ($F(1,35)=5.6$, $p<0.05$), but not of time ($F(1,35)=0.33$, $p=0.57$). The interaction terms were not significant.
ANOVA of % changes over time showed no differences between groups ($A\beta_{1-42}$: $F(1,36)=0.29$, $p=0.59$; tau: $F(1,36)=0.08$, $p=0.78$; ptau: $F(1,35)=0.12$, $p=0.73$). T-test of % changes showed increases for $A\beta_{1-42}$ and tau (10% and 7%, $p<0.05$) but not for ptau ($p=0.48$).

Discussion

We found that CSF $A\beta_{1-42}$ and tau, but not ptau-181 levels increased over time in a cohort of memory clinic patients, with comparable change in all diagnostic groups. In contrast with our expectation however, the cross-sectional difference between groups, exceeded by far the longitudinal changes within groups. We interpret these results as that repeated assessment of these biomarkers is currently not useful in a clinical setting, as they are insensitive to disease progression over a two year period.

The concept of critical difference provides a frame to evaluate the biological significance of repeated measurements of biomarkers. A change in serial results obtained for an individual is due not only to deterioration or improvement, but also to analytical sources of variation and within-subject random biological variation around the homeostatic set point.[26] The critical differences for $A\beta_{1-42}$, tau and ptau-181 in this study were 28%, 30% and 22%. Most percentage changes of the individual patients did not exceed the critical difference. The number of patients who did exceed the critical difference were equally divided among the patient groups. Of these, most increased over time, but some decreased. This supports our idea that the observed increase of CSF biomarkers over time does not seem to reflect the clinical course of the disease.

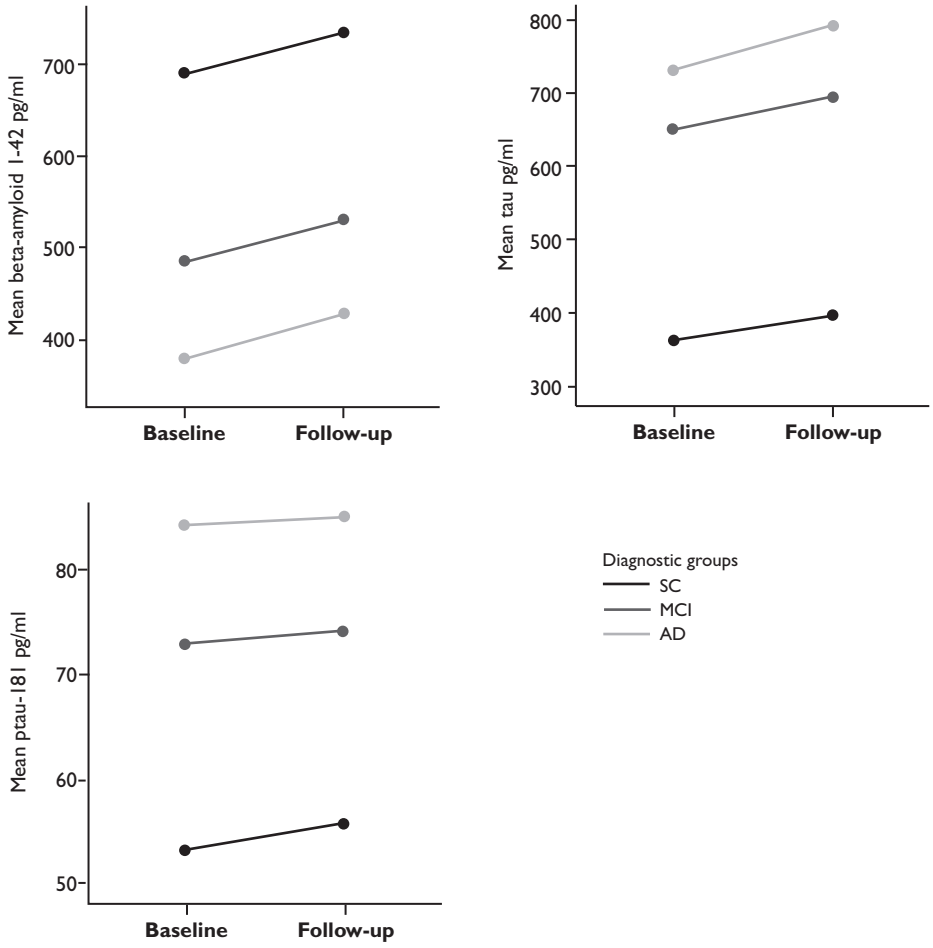

Figure 2. *Mean baseline and follow up $A\beta_{1-42}$, tau and ptau-181 levels for patient with subjective complaints, mild cognitive impairment and Alzheimer's disease*

SC=subjective complaints, MCI=mild cognitive impairment, AD=Alzheimer's disease. Mean baseline and follow-up beta-Amyloid$_{1-42}$, tau and ptau-181 (=phosphorylated tau at threonine-181) for patients with subjective complaints, mild cognitive impairment and Alzheimer's disease. Each panel represents a different biomarker. The figure shows, that between group differences exceed the within group differences over time. Furthermore, all lines are parallel, illustrating that change over time is comparable between groups.

Only small studies (sample sizes between 19 and 53) reported longitudinal changes of $A\beta_{1-42}$, tau or ptau levels in CSF, mainly in AD, while most studies assessed one ore two biomarkers only.[8-15] The majority showed no changes of these CSF biomarkers over time.[8,11,12,14] One study showed that $A\beta_{1-42}$ levels decrease with 10% over time,[15]

a second study described a 27% increase of tau level with stable $A\beta_{1-42}$ level,[13] and a third study showed stable $A\beta_{1-42}$ and tau levels but a 16% increase of ptau over time.[10] Few small studies described longitudinal levels of CSF biomarkers in MCI patients,[11,12] while especially this group of patients is of interest for early therapeutic intervention. In this study we describe longitudinal measurements of CSF biomarkers in a substantial cohort of memory clinic patients including patients with MCI and subjective complaints in addition to patients with AD. Although the subjective complaints group is relatively small, and our conclusions need some caution in this respect, inclusion of this patient group also is one of the strengths of our study.

The consistent increases of the CSF biomarkers across all diagnostic groups raise suspicion that other sources of variation exist that may explain these results. We were careful to avoid the influence of inter-assay variability by running baseline and follow-up samples in the same assay at the time of the second LP. Difference in follow-up time and thus storage time of CSF-samples, however, might have caused an increase in CSF biomarkers. For example different $A\beta_{1-42}$-forms might degrade after several years of storage at -80°C. Yet in a previous study on usefulness of longitudinal measurements of $A\beta_{1-42}$ levels we did not find an association for follow-up time and differences in repeated baseline $A\beta_{1-42}$-level confirming our earlier data in a study on effects of processing and storage conditions on $A\beta_{1-42}$-levels.[25,30] Another possible source of variation is the logistic limitations of the ELISA assays. Baseline samples were systematically placed before follow-up samples on the assay which might have caused a systematic error. We consider this unlikely, however, as the time delay for adding the reagents is too minimal to yield the increase we found. Moreover, this does not explain the fact that some patients decrease and some increase over time. Another possible explanation is the fact that all patient groups, also the subjective complaints group, might contain preclinical AD, simply on the basis that their referral to the memory clinic would increase the likelihood, through a selection bias, of their future development of AD. The presence of plaques and tangles in the brain would then cause a similar increase across all patient groups. This does not explain, however, that the longitudinal increase of biomarker levels are dwarfed by the cross-sectional group differences. Moreover, with the observed lower $A\beta_{1-42}$ and higher tau and ptau-181 in the AD group, one would expect $A\beta_{1-42}$ to decrease and tau and ptau-181 to increase in the subjective complaints and MCI group. Another source of variation is the time of day lumbar puncture was conducted. We did not choose a fixed time of the day for lumbar puncture. Patients were punctured between 9 AM and 4 PM. It is not likely that this generates a systematic increase of CSF biomarkers in all patient groups.

Cross-sectionally, CSF biomarkers can differentiate patients with AD from control subjects with relatively high accuracy.[3] Moreover, we recently demonstrated a cross-sectional relation between CSF tau levels and neuropsychological test performance in MCI patients.[31] The present longitudinal study elaborated on these findings, as we set out to relate cognitive decline over time with changes in CSF levels. We were not able to demonstrate such an effect, as change in CSF levels over time was similar across diagnostic groups, while the change in cognitive status was not: cognitive decline was evident in AD and MCI patients, but could not be observed in patients with subjective complaints. So the observed changes over time in CSF biomarkers do not seem to reflect the cognitive changes in these patient groups.

The reason that CSF biomarkers do not seem to reflect disease progression over time is not clear. Possibly, pathological CSF biomarker levels reflect a threshold phenomenon: as soon as histopathological changes in the brain occur, levels become pathological, and do not change over time. Since pathological changes of AD may occur as early as 20 to 30 years before clinical diagnosis, the MCI stage may be too late in the course of the disease to detect a transition from normal to pathological CSF biomarker levels. This study included a sample of patients with subjective complaints, supposedly a clinical stage before MCI, but we were not able to demonstrate such a transition during the period under study. Moreover, cross-sectionally, one would expect a bi-modal distribution of CSF biomarker level. The continuous distribution that is in fact observed does not support the idea of a threshold phenomenon.

Future studies are needed to optimize standardization of assays, thereby reducing the variability and increasing the sensitivity to change over time. Alternatively, it is conceivable that other CSF biomarkers, like inflammatory chemokines, serum biomarkers, or markers yet to be defined, are more suitable for monitoring progression of disease.[32,33]

Acknowledgements

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Chapter 3

CSF biomarkers in young and old AD patients

CSF biomarker levels in early and late onset Alzheimer's disease

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Neurobiology of Aging in press

Abstract

Objective: To compare CSF levels of beta-Amyloid$_{1-42}$ (Aβ_{1-42}), total tau (tau) and tau phosphorylated at threonine-181 (ptau-181) between AD patients and controls according to age.

Methods: 248 AD patients (48% men) and 127 controls (51% men, 22 volunteers and 105 subjective complainers) underwent lumbar puncture. Both patients and controls were divided into a young (<65 years) and old (≥65 years) group.

Results: All three biomarkers showed main effects of diagnosis ($p<0.001$). There was an interaction between diagnosis and age for all three biomarkers ($p<0.05$), as old controls had lower Aβ_{1-42} and higher (p)tau than young controls (Aβ_{1-42} 699±250 versus 866±191 pg/ml, tau 408±245 versus 243±102 pg/ml, ptau-181 60±28 versus 42±15 pg/ml), but there was no difference according to age among AD patients (Aβ_{1-42} 451±178 versus 425±146 pg/ml, tau 741±460 versus 798±467 pg/ml, ptau- 91±42 versus 91±41 pg/ml).

Conclusion: We found that the older control group had lower Aβ_{1-42} and higher (p) tau compared to the younger control group. This suggests that older individuals may have AD pathology, even in the absence of objective cognitive impairment.

Introduction

Alzheimer's disease (AD) is the most common form of dementia. The neuropathological hallmarks of AD are neuritic plaques, mainly composed of beta-Amyloid$_{1-42}$ (Aβ_{1-42}), and neurofibrillary tangles containing (phosphorylated) tau. There is evidence that CSF levels of Aβ_{1-42} and tau reflect the presence of the neuropathological hallmarks of AD from neuropathological and PIB-PET studies.[1-3] In addition, CSF levels of Aβ_{1-42} and (phosphorylated) tau have been shown to discriminate AD from controls with reasonable accuracy, although biomarker levels do overlap between groups.[4;5]

The term AD was originally reserved for individuals with presenile onset of symptoms, whereas the expression senile dementia was used when onset was after 65 years of age.[6] Later in history these disorders were held to represent a single, homogeneous entity, as it was observed that plaques and tangles are present in the brains of patients with both early and late onset AD.[7] However, post mortem studies have shown that in young patients with AD there is a strong correlation between dementia severity and plaque and tangle burden, whereas this association is not found in elderly patients.[8] Furthermore, plaque and tangle load as well as the cholinergic deficits may be more severe in young than in old AD patients.[9;10] Therefore, one would expect differences in CSF biomarker levels for young and old AD patients.

Few studies have analysed differences in CSF biomarker levels in patients with early versus late onset AD and came up with inconsistent results.[11-15] The aim of this study was to compare CSF levels of Aβ_{1-42}, total tau (tau) and tau phosphorylated at threonine-181 (ptau-181) between AD and controls according to age with the ultimate goal to gain further insight in possible differences of AD pathology in young and old patients.

Methods

Study population

248 patients with Alzheimer's disease and 127 control subjects (22 volunteers without cognitive complaints and 105 patients with subjective complaints) underwent lumbar puncture (LP) at the Alzheimer Center of the VU University Medical Center (VUMC) between November 2000 and December 2006. All patients underwent a standardized clinical assessment, including medical history, physical and neurological examination including Mini Mental State Examination (MMSE),[16] laboratory tests, psychometric evaluation, EEG and brain MRI. Diagnosis at time of lumbar puncture

was made according to the clinical criteria of NINCDS-ADRDA for probable AD[17] by a multidisciplinary team of neurologists, neuropsychologists, a neurophysiologist, a psychiatrist, a geriatrician and a radiologist. When all clinical investigations were normal, patients were considered to have subjective complaints. In addition, 22 subjects without cognitive complaints volunteered to participate in the study. Clinical follow-up data were available for 55 controls (35 young, 20 old; mean follow-up time 1.8, range 0.34–5.18) including 52 MMSE.[17-20] For AD patients 144 follow-up MMSE were available. The diagnosing team was not aware of the results of the CSF analysis. The study was approved by the ethical review board of the VUMC and all subjects gave written informed consent.

CSF analysis

CSF was obtained by LP between the L3/L4 or L4/L5 intervertebral space, using a 25-gauge needle, and collected in 12-mL polypropylene tubes. Within two hours, CSF samples were centrifuged at 2100*g* for 10 minutes at -4°C. A small amount of CSF was used for routine analysis, including total cells (leucocytes and erythrocytes), total protein and glucose. CSF was aliquoted in polypropylene tubes of 0.5 or 1 ml, and stored at -80°C until further analysis. CSF $A\beta_{1-42}$, tau and ptau-181 were measured by commercially available sandwich ELISAs (Innotest beta-Amyloid$_{1-42}$,[21] Innotest hTau-Ag,[22] and Innotest Phosphotau $_{(181P)}$,[23] Innogenetics, Ghent, Belgium). The team involved in the CSF analysis was not aware of the clinical diagnoses. The inter-assay coefficient of variation for $A\beta_{1-42}$ was 14%, 12% for tau and 9% for ptau-181.

Statistical analysis

For statistical analysis, SPSS version 12.0 (Chicago IL) was used. Frequency distributions for sex were compared with chi-squared tests. Student's T-test was used to compare age and MMSE between groups. Both the patient and control group were arbitrarily divided into a young (younger than 65 years) and old (65 years and older) group. To assess the respective effects of diagnosis and age on biomarker levels, we first used two-way Analysis of Variance (ANOVA) with diagnosis and age (<65 years vs ≥65 years) as factors. In addition, bivariate Spearman's correlation coefficients were calculated between age and MMSE and CSF biomarker levels for controls and AD patients separately. Finally, linear regression analyses were performed with CSF biomarker levels as dependent variables and diagnosis, age and the interaction term of diagnosis and age as independent variables.

Results

Demographic characteristics are represented in table 1. No differences were found for sex between young and old AD patients and controls. Age showed no differences in the old group, but in the young group patients were slightly older than controls ($p<0.01$). Of the 55 controls with clinical follow-up, six showed clinical progression (4 mild cognitive impairment (MCI) (one young, three old controls), one AD and one frontotemporal lobar degeneration (both young controls)). Young and old controls had comparable cognitive function at baseline, but annual change in MMSE showed more decline in old controls compared to young controls ($p<0.01$). Young AD patients had lower baseline MMSE scores than old AD patients, and more cognitive decline by annual MMSE change ($p<0.05$).

Table 1. *Demographic characteristics of AD patients and controls according to age*

	Young		Old	
	Controls	**AD**	**Controls**	**AD**
N	82	103	45	145
Age	55 (6)	57 (4)*	73 (5)	73 (5)
Gender, M/F	41/41	47/56	24/21	73/72
Baseline MMSE [a]	29 (1)	20 (6)#	29 (1)	21 (5)
Annual MMSE change[b]	0.5 (0.3)§	-3.0 (3.8)#	-1 (1.8)	-1.8 (3.2)

Data are presented as mean (standard deviation) unless indicated otherwise.
AD=Alzheimer's disease, MMSE=mini mental state exam.
[a] missing for 7 controls and 6 patients.
[b] available for 52 controls and 144 patients.
For age, students t-tests were performed between young patients and young controls as well as between old patients and old controls. For MMSE, t-tests were performed between young and old controls and between young and old patients. Chi squared tests were used for gender comparisons.
* significant difference for pairwise comparison between patients and controls ($p<0.01$)
# significant difference for pairwise comparison between young and old patients ($p<0.05$)
§ significant difference for pairwise comparison between young and old controls ($p<0.01$)

Two-way ANOVA revealed main effects of diagnosis for all three biomarkers ($p<0.001$). A main effect of age was found for $A\beta_{1-42}$ and ptau-181 ($p<0.05$), but not for tau ($p=0.20$). There was an interaction between diagnosis and age for all three biomarkers ($p<0.05$), as young controls had higher $A\beta_{1-42}$ and lower (p)tau compared to old controls, but there was no difference according to age among AD patients (see also figure 1). In table 2 mean biomarker levels are presented. Pairwise comparisons of young AD patients with controls and old AD patients with controls showed that both young and old patients

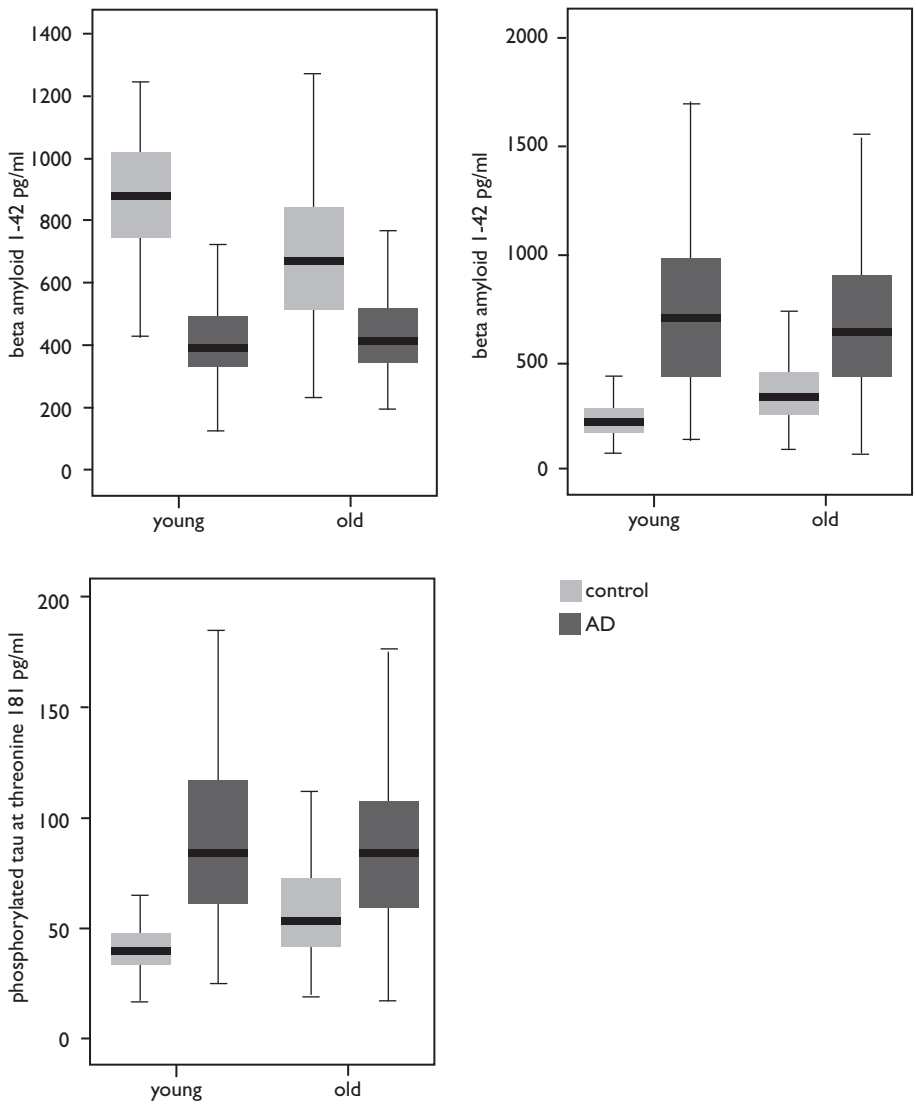

Figure 1. *Biomarker levels for young and old AD patients and controls*

Box plots of CSF biomarker levels by group. Boxes represent the median, the 25th and the 75th percentiles, bars indicate the range of data distribution. Two-way ANOVA revealed main effects of diagnosis for all three biomarkers ($p<0.001$, table 1). A main effect of age was found for $A\beta_{1-42}$ and ptau-181 ($p<0.05$), but not for tau ($p=0.20$). There was an interaction between diagnosis and age for all three biomarkers ($p<0.05$), as young controls had higher $A\beta_{1-42}$ and lower (p)tau compared to old controls, but there was no difference according to age among AD patients.

had lower $A\beta_{1-42}$ and higher tau and ptau-181 levels. Pairwise comparisons of young and old AD patients showed similar CSF biomarker levels, while young controls had higher $A\beta_{1-42}$ and lower tau and ptau-181 than old controls. In addition, we calculated the ratio of tau / $A\beta_{1-42}$, and found an effect for diagnosis ($p<0.01$) and the interaction between diagnosis and age ($p<0.05$), but not for age ($p=0.30$).

Table 2. *Biomarker profile of AD patients and controls according to age*

	Young		Old	
	Controls	**AD**	**Controls**	**AD**
$A\beta_{1-42}$	866 (191)	425 (146)*	700 (250)#	451 (178)*
Tau	243 (102)	798 (467)*	408 (245)#	741 (460)*
Ptau-181	42 (15)	91 (41)*	60 (28)#	91 (42)*
Tau / $A\beta_{1-42}$	0.29 (0.14)	2.1 (1.4)*	0.74 (0.77)#	1.9 (1.6)*

Data are presented as mean (standard deviation) unless indicated otherwise.
AD=Alzheimer's disease, $A\beta_{1-42}$=beta-Amyloid$_{1-42}$, tau=total tau, ptau-181=phosphorylated tau at threonine-181.
Two-way ANOVA revealed main effects of diagnosis for all three biomarkers ($p<0.001$). A main effect of age was found for $A\beta_{1-42}$ and ptau-181 ($p<0.05$), but not for tau and tau / $A\beta_{1-42}$ ($p=0.2$ and $p=0.3$). There was an interaction between diagnosis and age for all three biomarkers and tau / $A\beta_{1-42}$ ($p<0.05$).
significant difference for pairwise comparison between young and old controls ($p<0.01$)
* significant difference for pairwise comparison between patients and controls ($p<0.01$)

There were significant associations between CSF biomarker levels and age in the control group ($A\beta_{1-42}$: $r_s=-0.26$, tau: $r_s=0.46$, ptau-181: $r_s=0.42$, tau / $A\beta_{1-42}$: $r_s=0.53$; all $p<0.01$), but not in the patient group (all $p>0.10$). In AD patients, baseline MMSE was weakly but significantly correlated with CSF $A\beta_{1-42}$ ($r_s=0.16$, $p<0.05$) and tau / $A\beta_{1-42}$ ratio ($r_s=-0.17$, $p<0.01$) but not with tau or ptau-181. In the control group no associations were found between baseline MMSE and CSF biomarkers.

Finally, we performed linear regression analyses with CSF biomarkers as dependent variables. For all biomarkers there were main effects of diagnosis ($A\beta_{1-42}$ β(SE)=-910(132), tau β(SE)=1025(279), ptau-181 β(SE)=81(26); $p<0.005$)) and age ($A\beta_{1-42}$ β(SE)=-7(2), tau β(SE)=8(3), ptau-181 β(SE)=1(0.3); $p<0.05$)). For $A\beta_{1-42}$ and tau there was an interaction between diagnosis and age ($A\beta_{1-42}$ β(SE)=9(2), tau β(SE)=-9(4); $p<0.05$), but not for ptau-181 ($p=0.11$). Figure 2 illustrates the lack of association between CSF levels and age in AD patients, as the regression lines are almost horizontal, while the regression lines of the controls show a clear slope for all three CSF biomarkers.

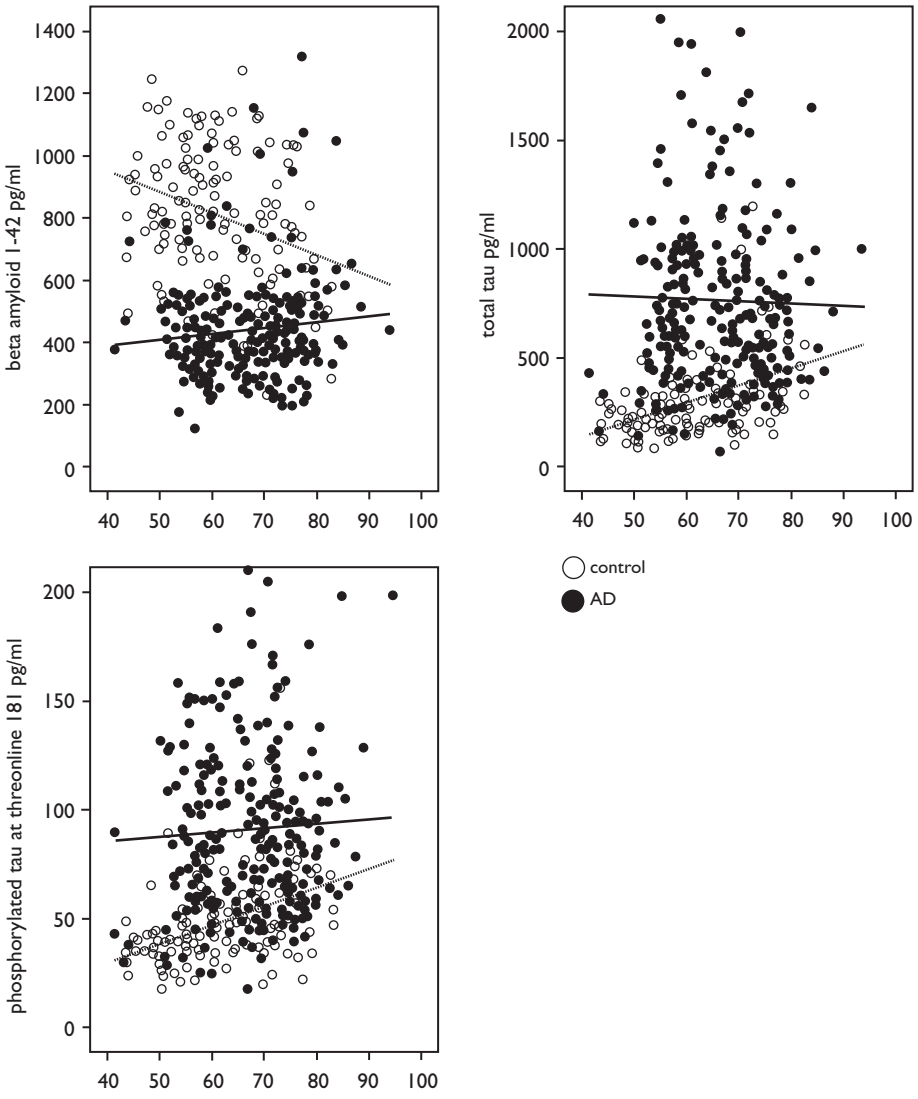

Figure 2. *Biomarker levels of AD patients and controls according to age*

Scatter plots of CSF biomarker levels by age. AD patients are represented by open circles, controls by closed circles. The converging regression lines indicate that CSF biomarker levels are differently affected by age in controls compared to AD patients. For all three biomarkers there were main effects of diagnosis ($A\beta_{1\text{-}42}$ β(SE)=-910(132), tau β(SE)=1025(279), ptau-181 β(SE)=81(26); p≤0.005)) and age ($A\beta_{1\text{-}42}$ β(SE)=-7(2), tau β(SE)=8(3), ptau-181 β(SE)=1(0.3); p<0.01)). For $A\beta_{1\text{-}42}$ and tau there was an interaction between diagnosis and age ($A\beta_{1\text{-}42}$ β(SE)=9(2), tau β(SE)=-9(4); p<0.05), but not for ptau-181 (p=0.11).

Discussion

We found that the difference in CSF biomarker levels between young AD patients and controls is larger than the difference between old AD patients and controls. As CSF biomarker levels between young and old AD patients were similar, this was attributable to the old control group having lower $A\beta_{1-42}$ and higher (p)tau compared to the young control group.

Few previous studies compared CSF biomarker levels in young and old AD patients. One study found lower $A\beta_{1-42}$ levels in old AD patients, another study found tau to be more increased in young AD patients.[11;13] Other studies did not find a difference for biomarker levels between young and old AD patients, which is in accordance with our study.[12;14;15] Remarkably, in spite of the age effect in controls, young patients seem to have similar abnormal levels as old patients, and are already at the bottom level.

Like CSF biomarkers, atrophy of the medial temporal lobe (MTA) on MRI has been found to be an early and sensitive marker for AD and is assumed to reflect underlying neuronal loss of the hippocampus and temporal lobe.[24] In the early stages of AD, however, absence of MTA does not exclude AD and especially young AD patients often lack prominent MTA.[25] In a study comparing young and old AD patients with age matched controls both old patients and old controls were found to have more MTA than young patients and controls, suggesting an additive effect of the factors age and diagnosis.[26] This is in contrast with our CSF biomarkers study, where a clear age effect was observed among controls, but both young and old AD patients had comparably abnormal biomarker levels. This suggests that especially in young patients, CSF biomarkers have more discriminative value than MRI markers.[27]

Several studies question the scientific basis for combining AD at young and old age as one homogeneous entity. In young patients with AD there is a strong correlation between dementia severity and plaque and tangle burden, whereas this association has not been found in elderly patients with the disease.[8] In contrast, the degree of concomitant cerebrovascular pathologic findings is more severe in older AD patients. Therefore, it has been suggested that younger AD patients have a 'pure' and possibly genetic origin of AD while older AD patients more often have a multifactorial origin of dementia caused by a combination of genetic and environmental factors such as age-related changes, cerebrovascular changes and Alzheimer pathology.[29] The similar CSF biomarker levels for young and old AD patients we found, may suggest similar AD pathology in young and old AD patients.[2;30;31] However, since asymptomatic individuals at older age seem to have lower CSF $A\beta_{1-42}$ and higher CSF tau and ptau-181 levels, our study suggests a higher discriminative value of CSF biomarkers in young compared to

old patients. This was supported by a larger area under the curve in ROC curves for the young group compared to the old group for all three CSF biomarkers (results not shown). On the one hand this may imply that age-dependent reference values should be used, when applying CSF analysis in clinical practice. Alternatively, one set of reference values may be used irrespective of age. At older age, there is more overlap in CSF biomarker levels between controls and patients, which we feel stresses the need for careful follow up of older subjects with abnormal CSF biomarkers.

Former studies describing CSF biomarker levels in healthy individuals found conflicting results.[12;32;33] In a study with 231 healthy individuals Sjogren et al. found CSF tau but not $A\beta_{1-42}$ to be age dependent.[32] By contrast, Peskind et al found CSF $A\beta_{1-42}$ to decrease with age especially in ApoE4 positive subjects, while others found an increase.[33-36] We found a clear age effect among our controls for all three biomarkers, as we observed more abnormal CSF levels with increasing age. A possible limitation of this study is the fact that the control group for a large part comprised patients with subjective complaints. These patients are known to have a higher risk of developing AD in the future.[37-39] However, patients with subjective complaints were equally distributed over the young and old control group. This suggests that the more pathological levels in the old control group are not (only) explained by the higher risk of pre-clinical AD in patients with subjective complaints, but more so by the fact that older individuals in general are more prone to have amyloid plaques with neurofibrillar changes and are at higher risk of developing AD.[40] These findings are congruent with former studies, in which an increased ratio of CSF tau / $A\beta_{1-42}$ or reduced CSF $A\beta_{1-42}$ level has been described to be predictive of cognitive decline in nondemented older adults, suggesting that CSF biomarkers can detect the underlying disease process even during the pre-clinical stage.[41-44] Moreover, previous post mortem and animal studies, described an increase of plaques and tangles and beta-Amyloid with age in non demented individuals and wild type mice.[40;45;46] The abnormal CSF $A\beta_{1-42}$ and (p)tau levels in our controls may therefore suggest presence of AD pathology in older individuals, and although we lack neuropathological information about our control group, it is supported by the observed annual MMSE decrease in the older control group.

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Chapter 4

Biomarkers for Alzheimer's disease in post mortem CSF

Post mortem CSF beta-Amyloid$_{1-42}$, tau and phosphorylated tau in Alzheimer's and Lewy body disease

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Abstract

Objective: To describe post mortem CSF biomarker levels of beta-Amyloid$_{1-42}$ (Aβ_{1-42}), total tau (tau) and phosphorylated tau at threonine-181 (ptau-181) in patients with Alzheimer's (AD) and Lewy body disease (LBD) versus controls.

Methods: 17 AD patients, 20 LBD patients and 10 controls underwent cisternal puncture during autopsy. Post mortem CSF levels of Aβ_{1-42}, tau and ptau-181 were measured.

Results: Levels of tau and ptau-181 were extremely high compared to intra vitam levels, while levels of Aβ_{1-42} were extremely low. No differences were found between diagnostic groups. Both CSF levels of tau and ptau-181 were correlated with time between death and cisternal puncture.

Conclusion: Post mortem CSF levels of tau, ptau-181 and Aβ_{1-42} are not interpretable. Most likely this is due to massive neuronal cell death, and does not accurately reflect intra vitam neuropathology. Hence these biomarkers cannot be used in post mortem CSF for discrimination of neurodegenerative disorders.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia. The clinical diagnosis of AD is made with clinical criteria, that largely depend on the exclusion of other dementias.[1] Dementia with Lewy Bodies (DLB) is regarded by some as the second most common cause of dementia.[2] The term Lewy Body Disease (LBD) includes the clinical spectrum of Parkinson's disease (PD), Parkinson's disease with dementia (PDD) and dementia with Lewy bodies (DLB).[3] The diagnostic accuracy of the clinical criteria of both AD and the clinical spectrum of LBD is relatively low, with sensitivity of around 80% and specificity of 70%.[2;4-6] The definite diagnosis of AD and LBD can only be made by neuropathology, which is regarded as the gold standard.[1;2;6]

Since CSF is in direct contact with brain tissue, it is argued that it reflects neuropathology. Intra vitam CSF biomarker levels of beta-Amyloid$_{1-42}$ (Aβ_{1-42}) and (phosphorylated) tau have been shown to differentiate AD from controls or PD with reasonable accuracy.[7-9] Tau phosphorylated at threonine-181 (ptau-181) has been suggested to discriminate AD from DLB and PD from PDD, although overlap occurs between groups, which is consistent with the clinical overlap between AD and DLB and the neuropathological overlap between PD, PDD and DLB.[10-12]

Few studies described biomarkers in post mortem CSF, showing no differences between AD and controls.[13;14] In contrast to intra vitam CSF, post-mortem CSF may yield additional information reflecting neuropathology. Brain cell necrosis, however, may influence protein concentrations such as (phosphorylated) tau related to brain damage and neurodegeneration.[15] The aim of this study was to describe post mortem CSF biomarker levels of Aβ_{1-42}, tau and ptau-181 in AD and LBD patients versus controls.

Methods

Study population

Between July 1994 and August 2003 post mortem ventricular CSF was collected from 17 patients with AD, 20 patients with LBD and 10 controls at the department of neurology of Medisch Spectrum Twente Hospital Group in Enschede (The Netherlands). The LBD patients in this study were described in a previous study on correlation of cognitive status with neuropathologic stage in PD and included 18 patients with PD and 2 with PDD.[3] All autopsies were performed in the same department by an experienced

pathologist (RAIdV). AD pathology was rated using an established rating system for cortical neurofibrillary changes of the Alzheimer type and the CERAD criteria. [16;17] Lewy body pathology was rated using the consensus neuropathological criteria of Mc Keith et al.[2]

Immunocytochemistry and staining

Sections of 6 µm thickness underwent immunocytochemical treatment with a monoclonal antibody against α-synuclein (NCL-L-ASYN, 1:60; Novocastra, Newcastle-upon-Tyne, UK), Sections were counterstained with hematoxilin. The Gallyas silver iodide method was used to visualize neurofibrillary changes of the Alzheimer type. Finally, immunoreactions with antibodies against abnormally phosphorylated tau protein (AT8, 1:2000; Innogenetics, Ghent, Belgium) and a monoclonal antibody against aggregated beta-Amyloid protein (M 0872, 1:150; Dako, Hevelee, Belgium) were performed.

CSF analysis

CSF was obtained by puncturing the pontomedullary or cerebellomedullary cisterns using a 22 Gauge needle, and collected in 12-mL polypropylene tubes. Within one hour, CSF samples were centrifuged at 700 *g* for 10 minutes at room temperature. CSF was aliquoted in polypropylene tubes of 0.1 ml, and stored at -80˚C until further analysis. CSF $A\beta_{1-42}$, tau and ptau-181 were measured by commercially available sandwich ELISAs (Innotest beta-Amyloid$_{1-42}$,[18] Innotest hTau-Ag,[19] and Innotest Phosphotau $_{(181P)}$,[20] Innogenetics, Ghent, Belgium). The team involved in the CSF analysis was not aware of the diagnoses. The inter-assay coefficient of variation for $A\beta_{1-42}$ was 14%, 12% for tau and 9% for ptau-181.

Statistical analysis

For statistical analysis, SPSS version 14.0 (Chicago IL) was used. Frequency distributions for sex were compared with chi-squared tests. One way Analysis of Variance (ANOVA) with post hoc t-tests were used to assess differences between diagnostic groups in age and time between death and cisternal puncture. Kruskal Wallis test with diagnosis as grouping variable was used to assess differences in biomarker levels for the three diagnostic groups. In addition, correlations between CSF biomarker levels and time between death and cisternal puncture were assessed using Spearman's correlation coefficient (r_s).

Results

Demographic characteristics are presented in table 1. AD patients were older than controls. There was no difference in age between AD and LBD patients or controls and LBD patients. No gender differences were found between diagnostic groups. Time between death and cisternal puncture was longer in controls compared to AD patients.

Table 1. *Demographic characteristics of patients and controls*

	AD	LBD	Controls
Age	81 (8)**	75 (6)	72 (10)
Gender: M/F	5/12	9/11	7/3
Time between death and cisternal puncture (hours)	20 (8)*	27 (15)	36 (20)

Data are presented as mean (standard deviation) unless indicated otherwise.
AD=Alzheimer's disease, LBD= Lewy body disease.
**AD patients > controls (p=0.01)
* AD patients < controls (p<0.05)

Neuritic plaque densities were normal according to CERAD criteria in all ten controls and twenty LBD patients, while two AD patients had probable and fifteen definite AD. Neurofibrillary changes met criteria for Braak stage I in six controls and ten LBD patients, Braak stage II in two controls and ten LBD patients, Braak stage III in two controls and one AD patient, Braak stage IV in two AD patients, Braak stage V in ten AD patients and Braak stage VI in four AD patients. All LBD patients met consensus neuropathological criteria of McKeith et al.[2]

Table 2 shows CSF biomarker levels in the different diagnostic groups. Levels of tau and ptau-181 were extremely high, while $A\beta_{1-42}$ was immeasurably low in all but two LBD patients. No differences were found for biomarker levels between diagnostic groups.

Across groups, CSF tau and ptau-181 levels were associated (r_s 0.83, $p<0.01$). A significant correlation was found for time between death and cisternal puncture and both CSF tau and ptau-181 level (r_s 0.33 for tau and 0.36 for ptau, $p<0.05$). No correlation was found for either tau or ptau-181 and age. Since only two $A\beta_{1-42}$ levels were measurable, statistical analysis was not useful.

Table 2. *Post mortem biomarker levels for patients and controls*

	AD	LBD	Controls
$A\beta_{1-42}$	0 (0-0)	0 (0-299) [a]	0 (0-0)
Tau	38200 (7825-313500)	47950 (5025-625000)	158000 (42750-423000)
Ptau-181	2655 (1230-9350)	1785 (375-9700)	2670 (185-10000)

Data are presented as median (range).
AD=Alzheimer's disease, LBD= Lewy body disease, $A\beta_{1-42}$=beta-Amyloid$_{1-42}$, tau=total tau, ptau-181=phosphorylated tau at threonine-181
[a] Two patients had detectable $A\beta_{1-42}$; one patient had 169 pg/ml, the other 299 pg/ml

Discussion

We found extremely high levels of tau and ptau-181 in post mortem CSF compared to intra vitam CSF of AD and LBD patients and controls, while $A\beta_{1-42}$ levels were extremely low in all three groups. No differences were found for post mortem CSF biomarker levels between the three diagnostic groups. There was an association between (p)tau level and post mortem hours.

Tau and ptau-181 are neuronal proteins located within the cell. The extremely high levels of tau and ptau-181 in this post mortem CSF study are very likely to be explained by massive neuronal cell death, followed by release of intracellular proteins. This finding is in accordance with previous post mortem CSF studies showing elevated levels of intracellular proteins released due to necrosis of the brain after death.[15;21] This is corroborated by the correlation of time between death and lumbar puncture and both tau and ptau-181 levels in this study. It seems as though from the moment of death, brain cell necrosis is increasing, leading to increasing CSF levels of intracellular proteins. The extremely high levels of tau and ptau-181 are also in accordance with previous findings of high (p)tau levels in CJD, stroke and trauma reflecting considerable neuronal cell death in a short time period.[22-24]

In contrast to (p)tau, $A\beta_{1-42}$ levels were immeasurably low, which confirms a previous study describing post mortem CSF $A\beta_{1-42}$ levels.[25] Intra vitam $A\beta_{1-42}$ is an extracellular protein known for its tendency to form aggregates either in CSF or, as part of Alzheimer pathology, in plaques located in between neuronal cells. The extremely low $A\beta_{1-42}$ levels suggest that CSF $A\beta_{1-42}$ aggregates on a large scale after death, and that it is not recognized by the ELISA in its aggregated form. Another possible explanation for the extremely low $A\beta_{1-42}$ levels is that the $A\beta_{1-42}$ aggregates

have precipitated in plaques in the brain, and are not present anymore in post mortem CSF.

A possible limitation in this study is the fact that post mortem CSF was obtained by pontomedullary or cerebellomedullary cisternal puncture, while intra vitam CSF for evaluation of cognitive disorders is obtained by lumbar puncture. Some intra vitam studies observed that for brain derived proteins, such as tau, ventricular levels are higher than lumbar levels, thereby influencing the (p)tau load in total CSF volume.[26;27] We think, however, that the small increases in ventricular when compared to lumbar biomarker levels during life, are dwarfed by the extremely high post mortem levels of (p)tau. Proteins such as $A\beta_{1-42}$, that are not exclusively brain derived, had a reciprocal pattern intra vitam, showing lower levels ventricular and higher levels lumbar. This may correspond with the massive aggregation of $A\beta_{1-42}$ in plaques in the brain post mortally, thereby explaining the extremely low $A\beta_{1-42}$ levels.

During life ptau-181 discriminates AD from DLB and PD from PDD with reasonable accuracy.[10-12] Definite diagnosis of both AD and LBD can only be made at autopsy. This study showed in a sample of pathologically diagnosed patients, that discrimination between AD, LBD and controls is not possible with post mortem CSF, including ptau-181 level. This is very likely explained by the fact that the neuropathological differences between AD and LBD during life cannot be picked up by post mortem levels of (p)tau due to massive neuronal cell death. Therefore, we suggest that future autopsy studies should concentrate on intra vitam CSF biomarker levels related to neuropathological diagnosis, which is regarded as the golden standard. Only few previous studies assessed the relation of intra vitam obtained CSF to neuropathological diagnosis at autopsy, yielding a diagnostic accuracy of around 80%-90%.[28;29]

We conclude that, although CSF biomarkers $A\beta_{1-42}$, tau and ptau-181 are widely used during life, these biomarkers cannot be used for differentiation of AD, LBD and controls in post mortem CSF. Further research should concentrate on identification of new proteins in post mortem CSF as potential biomarkers of neurodegeneration and the correlation of intra vitam CSF biomarkers with post mortem neuropathological diagnosis.

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Chapter 5

Combination of CSF biomarkers and MRI in the diagnosis of Alzheimer's disease

CSF biomarkers and medial temporal lobe atrophy predict dementia in mild cognitive impairment

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Abstract

Objective: To study CSF biomarkers, beta-Amyloid$_{1-42}$ (Aβ_{1-42}) and tau, and medial temporal lobe atrophy (MTA) on MRI in their ability to predict dementia in patients with mild cognitive impairment (MCI).

Methods: 59 MCI patients (49% male, mean age 69±8), follow-up 19 months), were included. Baseline CSF levels of Aβ_{1-42}, tau and MTA-score were dichotomized.

Results: 33 (56%) of the MCI patients progressed to dementia, 30 of which had Alzheimer's disease. Lower CSF Aβ_{1-42} level, higher CSF-tau and higher MTA-scores at baseline were found in progressed patients. Cox proportional hazards models revealed that abnormal CSF Aβ_{1-42}, CSF tau and MTA were significantly associated with dementia at follow-up (hazard ratio (95% confidence interval): 4.0 (1.3-12.1), 5.9 (1.6-21.7) and 2.1 (1.0-4.6)). A fourfold higher risk was found for patients with both abnormal CSF biomarkers and MTA compared to patients with either test abnormal. 94% of patients with both abnormalities converted to dementia.

Conclusions: These findings suggest an added value of CSF to MRI in the diagnostic work up of patients presenting at a memory clinic.

Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly. Patients with mild cognitive impairment (MCI) do not have overt dementia and yet are not cognitively normal for age.[1;2] A substantial proportion of MCI patients will progress to dementia, mostly AD, during follow-up. Early identification of these patients that are not yet demented is important, because disease-arresting compounds (e.g. drugs that effect the deposition of beta-Amyloid$_{1-42}$) may have the greatest potential in the earliest stages of disease. Diagnosis of incipient AD in MCI patients on clinical grounds remains difficult. Hence, there is a great need for diagnostic markers in patients in the earliest phase of the disease.

The major histopathological hallmarks of AD are senile plaques, containing beta-Amyloid$_{1-42}$ (Aβ_{1-42}) and neurofibrillary tangles with microtubuli-associated tau protein. These pathological changes are present before the onset of clinical dementia.[3] Decreased Aβ_{1-42} levels and elevated tau protein levels in CSF can differentiate patients with AD from control subjects or patients with other neurological conditions with relatively high accuracy.[4] Moreover, these CSF changes can already be detected in patients with MCI who will progress to AD.[5;6]

In AD, the earliest neuropathological changes occur in the medial temporal lobe, including the hippocampus and parahippocampal gyrus. On MRI, medial temporal lobe atrophy (MTA) is a marker of AD that has been shown to be predictive of AD in MCI patients.[7] Neither CSF biomarkers nor MTA, however, achieve high enough diagnostic accuracy to predict AD in MCI patients. We aimed to study CSF biomarkers and MTA in their ability to predict dementia and to assess the predictive value of the combination of these biomarkers.

Methods

Study population

Sixty-four patients with MCI were consecutively recruited at the Alzheimer Centre of the VU Medical Centre (VUMC) between January 2001 and October 2004. Patients underwent a standardized clinical assessment, including medical history, physical and neurological examination including Mini Mental State Examination (MMSE),[8] laboratory tests, psychometric evaluation, EEG and brain MRI. At least one follow-up investigation was performed in all MCI patients. Follow-up time was defined as the time until conversion date (in case of progression to dementia) or until the last visit to the memory clinic (in case of stable MCI). The mean follow-up period for the cohort was 19 months

(range 4-45). The initial and follow-up diagnoses were made by a multidisciplinary team of neurologists, neuropsychologists, a neurophysiologist, a psychiatrist, a geriatrician and a radiologist. Criteria of Petersen et al. were used for MCI,[9;10] NINCDS-ADRDA criteria for AD,[11] criteria of Neary and Snowden for frontotemporal lobar degeneration (FTLD)[12] and NINDS-AIREN criteria for vascular dementia (VaD).[13] Although brain MRI contributed to the diagnostic process by excluding other neurological diseases (e.g. brain tumor), MTA scores were not used to select patients or diagnose MCI or AD. The team involved in the diagnostic work-up was not aware of the results of the CSF analyses. Median time between MRI and lumbar puncture (LP) was 2 months (range 0-12). The study was approved by the ethical review board of the VUMC and all subjects gave written informed consent.

CSF analysis

CSF was obtained by LP between the L3/L4 or L4/L5 intervertebral space and collected in polypropylene tubes. Within two hours, CSF samples were centrifuged at 3000 rpm for 10 minutes at 4°C. A small amount of CSF was used for routine analysis, including total cells (leucocytes and erythrocytes), total protein and glucose. CSF was aliquoted in polypropylene tubes of 0.5 or 1 ml, and stored at -80°C until further analysis. CSF $A\beta_{1-42}$ and tau were measured by commercially available sandwich ELISAs (Innotest beta-Amyloid$_{1-42}$,[14] Innotest hTau-Ag,[15] Innogenetics, Ghent, Belgium).

ApoE genotype

DNA was isolated from 10 ml EDTA blood. ApoE genotype was determined using the Light Cycler ApoE mutation detection method (Roche diagnositics GmbH, Mannheim, Germany).

MRI analysis

MRI scans were made on a 1.0 Tesla scanner (Siemens Magnetom Impact Expert, Erlangen, Germany) and included a coronal T1-weighted 3D inversion-prepared gradient echo-sequence (168 slices, FOV 250mm, matrix 256x256; slice thickness 1.5 mm, TE: 7ms, TR: 15 ms, TI 300 ms, flip angle 15 degrees). MTA was rated visually according to the method of Scheltens et al.[16] The score ranges from 0 (no atrophy) to 4 (severe atrophy). MTA scores of the left and right hippocampi were averaged. In one patient only the left MTA score was available because of an infarct in the right inferior temporal lobe. Two trained raters, who were blinded to clinical information, rated MTA. To determine reliability, 20 scans were scored twice (weighted Cohen's kappa for intra rater reliability >0.85 and inter rater variability >0.80).

Statistical analysis

For statistical analysis, SPSS version 12.0 was used. Differences between groups were analysed using Mann Whitney U test (continuous variables) or Chi-square test with continuity correction (categorical variables). CSF $A\beta_{1-42}$ was considered abnormal < 495 pg/mL and CSF tau was considered abnormal > 356 pg/mL(20). A CSF profile combining both $A\beta_{1-42}$ and tau was constructed, which was abnormal when both biomarkers were abnormal. An average MTA ≥1.5 was considered abnormal, indicating at least a score of 2 on one side.[7] ApoE genotypes were dichotomized on the basis of no or ≥1 ε4 allele. Cox proportional hazards models, that account for variability in length of follow-up, were used to assess the predictive value of CSF $A\beta_{1-42}$, CSF tau and MTA. Sex, age and ApoE genotype were entered as covariates. Hazard Ratios (HRs) are presented with 95% confidence interval (CI). Main outcome was progression to dementia, second outcome was progression to AD, excluding three cases who developed a different kind of dementia. Time to event curves were constructed with the Kaplan Meier method. In an additional Cox proportional hazards model, the additive risk of having both abnormal CSF profile and abnormal MTA over having only one of these abnormal biomarkers was assessed. Statistical significance was set at $p<0.05$.

Results

Three patients refused follow-up investigation and two patients died before follow-up investigation could take place. Of the remaining 59 MCI patients, 33 (56%) progressed to dementia, mostly AD (30 to AD; 2 to VaD and 1 to FTLD). There were 26 (44%) stable MCI patients. No significant differences were found between stable and progressive MCI patients for sex, age, follow-up time, ApoE genotype, baseline MMSE, and duration of symptoms (table 1). Significant differences were found for baseline CSF and MRI biomarkers, with the group of progressive MCI patients having lower CSF $A\beta_{1-42}$ levels, higher CSF tau levels and a higher MTA-score.

The survival curves for each biomarker are presented in figure 1. After correction for age, sex and ApoE genotype, Cox proportional hazards models revealed that abnormal CSF $A\beta_{1-42}$ yielded a fourfold and abnormal CSF tau a six-fold increased risk of progression to dementia (table 2). Subjects with an abnormal CSF profile were at a nearly threefold increased risk of progression to dementia. Abnormal MTA was associated with a twofold increased risk of progression to dementia. Risk estimates changed only marginally with AD as outcome.

Table 1. *MCI patients: characteristics and clinical variables*

	Stable	Progressive
N° of patients (M/F)	26 (15/11)	33 (14/19)
Age (SD)	69 (9)	70 (8)
Mean follow-up in months (range)	17 (5-36)	20 (4-45)
N° of ApoE4 positive patients	11 (42%)	23 (70%)
Baseline MMSE	26.5	25.4
Duration of symptoms in years (range)	2 (0-10)	2 (0-9)
$A\beta_{1-42}$ pg/mL (range)	547 (277-1450)	395 (178-1148)**
N° of patients <495 pg/ml	10 (38%)	26 (79%)**
Tau pg/mL (range)	426 (142-1340)	666 (260-3515)*
N° of patients >356 pg/ml	16 (62%)	29 (88%)*
MTA-score (SD)	0.8 (0.6)	1.5 (0.9)**
N° of patients >1.5	4 (15%)	19 (58%)**

Data are mean (sd) or n(%), unless indicated otherwise. SD=standard deviation; MMSE=Mini-Mental State Examination; $A\beta_{1-42}$= beta-Amyloid$_{1-42}$, MTA=medial temporal lobe atrophy. Disease duration, $A\beta_{1-42}$ and tau levels are median.
**p<0.01; *p<0.05

Table 2. *Risk estimates for abnormal biomarkers and progression to dementia*

Biomarker	All dementia n=59	AD n=56*
$A\beta_{1-42}$	4.0 (1.3 – 12.1)	5.0 (1.4 – 18.0)
Tau	5.9 (1.6-21.7)	5.3 (1.5-19.2)
CSF profile	2.8 (1.1-6.8)	3.0 (1.1-7.9)
MTA-score	2.1 (1.0 – 4.6)	2.3 (1.0 – 5.1)

Cox proportional hazards models with duration of follow-up as time variable and progression to dementia as dependent variable. Separate analyses were performed for each biomarker. Data presented as Hazard Ratio (HR) (95% CI). All HRs are adjusted for sex, age, ApoE. $A\beta_{1-42}$=beta-Amyloid$_{1-42}$. MTA=medial temporal lobe atrophy. $A\beta_{1-42}$ abnormal < 95 pg/mL. Tau abnormal >356 pg/mL. CSF profile: both abnormal $A\beta_{1-42}$ and abnormal tau. MTA-score abnormal ≥1.5.
*Vascular dementia (n=2) and frontotemporal lobe dementia (n=1) were not included in this analysis.

Because both CSF biomarkers and MTA are important predictors of dementia in MCI patients, we combined these biomarkers in an additional analysis. Table 3 shows biomarker profiles in stable and progressive MCI patients. Roughly one third of patients with a normal CSF profile and no MTA progressed to dementia, while half of patients with either an abnormal CSF profile or MTA progressed to dementia. Remarkably, patients with both an abnormal CSF profile and MTA were almost certain (94%) to progress to dementia. Formal analysis to assess the additive risk of having

both abnormal biomarkers over having only one abnormal biomarker showed a HR of 3.7 (95% CI 1.5-8.7) for progression to dementia and 4.0 (95% CI 1.6-10.2) for progression to AD. Thus, MCI patients with both abnormal CSF biomarkers and MTA have a fourfold higher risk of progression to dementia compared to patients with either one abnormality.

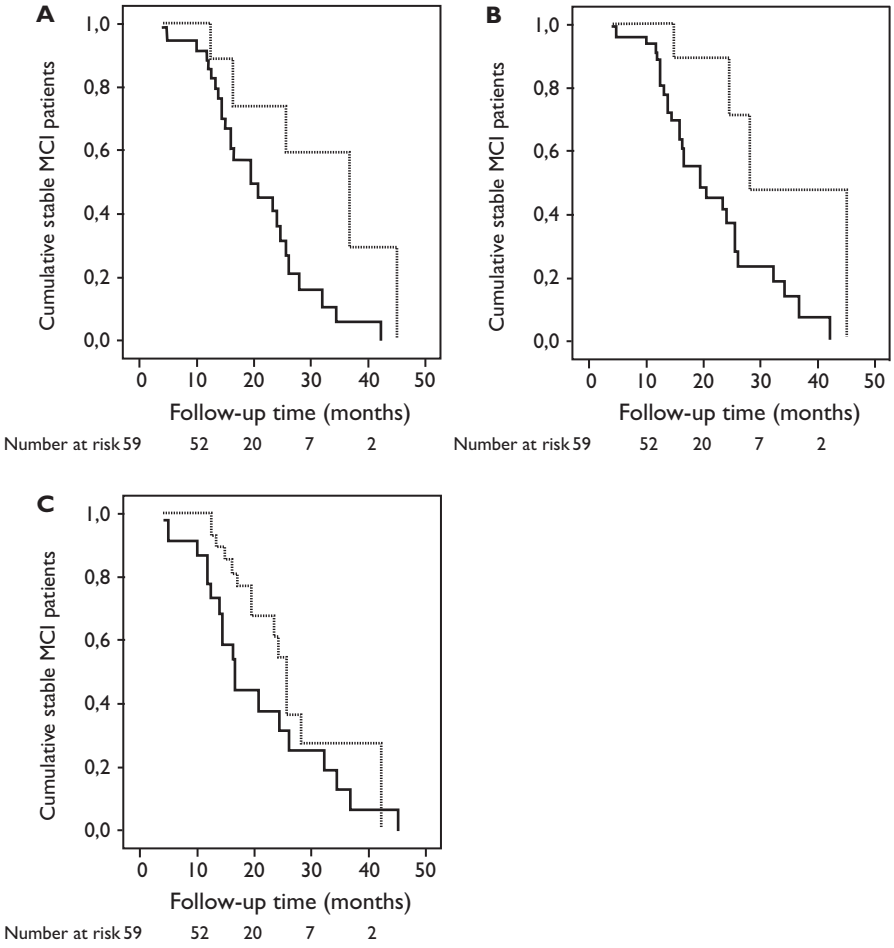

Figure 1. *Kaplan Meier – curves for CSF Aβ_{1-42} (A), tau (B) and MTA-score (C)*

Black lines indicate patients with abnormal biomarker values (Aβ_{1-42} <495 pg/mL, tau >356 pg/mL, averaged MTA-score ≥1.5), dotted lines indicate patients with normal biomarker values.

Table 3. *Biomarker profiles in stable and progressive MCI patients*

	Stable	Progressive	Total
CSF & MRI normal	14 (70%)	6 (30%)	20
CSF or MRI abnormal	11 (48%)	12 (52%)	23
CSF & MRI abnormal	1 (6%)	15 (94%)	16
Total	26	33	59

Abnormal $A\beta_{1-42}$ <495 pg/mL. Abnormal tau >356 pg/mL.
Abnormal CSF: both $A\beta_{1-42}$ and tau abnormal.
Abnormal MRI: MTA-score ≥ 1.5

Discussion

The main finding of this study is, that decreased CSF $A\beta_{1-42}$, increased CSF tau and an abnormal MTA-score are associated with a higher risk of progression to dementia, mostly AD. The predictive values of CSF biomarkers exceed that of the MTA-score. Moreover, MCI patients with both abnormal CSF profile and an abnormal MTA-score, were at a fourfold higher risk to progress to AD compared to patients with either an abnormal CSF profile or an abnormal MTA-score.

Our study confirms earlier findings of separate studies looking at either CSF biomarkers or MRI findings of MTA or hippocampal volumes in MCI patients who progress to dementia.[5-7;17-20] Our study is one of the very few that combined both types of biomarkers and assessed the combined value in a large sample of MCI patients with substantial follow-up. One study found abnormal CSF biomarkers in eight MCI patients, but did not find an additive effect of CSF biomarkers to the diagnostic accuracy of hippocampal volume.[21] Another study reported baseline CSF $A\beta_{1-42}$ level and total brain or ventricular volume to be associated in AD patients as well as in eight progressive MCI patients.[20] However, total brain or ventricular volume is not as specific for early AD pathology as MTA.[22]

Our data demonstrate that both CSF biomarkers and abnormal MTA-score are predictive of progression to dementia, mostly AD. The predictive value of CSF biomarkers was three times higher than the predictive value of the MTA-score. Visual inspection of the survival curves of CSF $A\beta_{1-42}$, tau and the MTA-score suggests similar predictive values for the first 18 months follow-up. After 18 months, the curves seem to diverge: patients with a normal MTA-score at baseline were more likely to progress to dementia than patients with normal baseline CSF $A\beta_{1-42}$ and CSF tau. It is conceivable, that a repeated MRI after 18 months would have revealed MTA that

was not yet present at baseline. Our results therefore suggest that CSF biomarkers may predict progression to dementia earlier in the course of the disease. The lower risk estimate for the MTA-score may reflect that an abnormal MTA-score predicts a swift progression to dementia, while the three times higher risk estimates of CSF biomarkers reflects their predictive value at a longer term. This corresponds with the presumed neuropathological course of AD: plaque and tangle formation, resulting in abnormal CSF biomarkers, precede neuronal loss. Neuronal loss results in atrophy that, especially when located in the medial temporal lobe, may precede measurable cognitive impairment.[23] Accordingly we found no significantly different MMSE scores, while MTA-scores were significantly different between stable and converting MCI patients.

Among the strengths of this study is the relatively large cohort of MCI patients that was consecutively recruited in a memory clinic setting. All patients were assessed in a standardized way and diagnosed according to the criteria of Petersen.[9;10] Compared to the conversion rate of 12% per year reported by Petersen et al, the conversion rate of 56% in our group of MCI patients seems rather high. However, our results are comparable to the conversion rate of other memory clinics,[5;24] while the conversion rate reported by Petersen et al was found in a general community setting. Among our MCI patients were two patients who converted to VaD and one to FTLD. It might be argued that these three patients should be excluded from analysis, because MRI abnormalities, especially cerebrovascular lesions in VaD, were already visible at baseline. However, MCI is a clinical diagnosis, and at baseline all three patients fulfilled the clinical MCI criteria. We performed an additional analysis, restricting our sample to stable patients and patients who progressed to AD. This yielded a higher predictive value for CSF $A\beta_{1-42}$ and the MTA-score and lower for CSF tau, suggesting that abnormal CSF $A\beta_{1-42}$ and an abnormal MTA-score are more specific for AD than abnormal CSF tau. The predictive value of other CSF biomarkers, such as phosphorylated tau, in combination with MRI remains to be established in future studies.

Among the possible limitations is the fact that MRI is part of the diagnostic work up of MCI patients, e.g. to exclude other neurological diseases like brain tumor.[25] Using MRI during the diagnostic process may have slightly influenced our results, as the presence of MTA may unconsciously have influenced the physicians to a diagnosis of AD. We therefore cannot entirely exclude the possibility of selection bias resulting in an underestimation of the risk estimate of the MTA-score. However, our results are comparable with other studies of the predictive value of MTA for progression to dementia.[7;18]

Our findings may have important clinical implications, as they suggest that the ability to detect patients with MCI who are at risk for dementia, especially AD, will increase when CSF analysis is combined with measures of MTA. As such, next to structural neuroimaging, CSF analysis should be considered in the initial evaluation of memory clinic patients not yet meeting criteria of dementia.

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Whole-brain atrophy rate and CSF biomarker levels in MCI and AD: a longitudinal study

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Abstract

Objectives: To assess associations between cerebrospinal fluid (CSF) biomarker levels and MRI-based whole-brain atrophy rate in mild cognitive impairment (MCI) and Alzheimer's disease (AD).

Methods: We included 101 patients (47 AD, 30 MCI, 24 controls) who underwent lumbar puncture at baseline and repeat MRI. A subgroup of 49 patients underwent a second lumbar puncture. CSF levels of beta-amyloid$_{1-42}$ (Aβ_{1-42}), tau and tau phosphorylated at threonine-181 (ptau-181), and whole-brain atrophy rate were measured.

Results: Across groups, baseline Aβ_{1-42} and tau were associated with whole-brain atrophy rate. Adjusted for age, sex and diagnosis, we found no association between Aβ_{1-42} or tau, and whole-brain atrophy rate. By contrast, high CSF levels of ptau-181 showed a mild association with a lower whole-brain atrophy rate in AD but not in controls or MCI patients. Finally, whole-brain atrophy rate was associated with change in MMSE, but change in CSF biomarker levels was not.

Conclusions: Whole-brain atrophy rate and CSF levels of Aβ_{1-42}, tau or ptau-181 provide complementary information in patients with MCI and AD.

Introduction

Both cerebrospinal fluid (CSF) biomarkers and magnetic resonance imaging (MRI) are increasingly used to detect and characterise brain changes associated with Alzheimer's disease (AD) in vivo. In CSF, decreased $A\beta_{1-42}$ levels and increased tau, and ptau-181 levels are thought to reflect the presence of AD pathology.[1] These CSF biomarkers have been shown to differentiate patients with AD from control subjects with reasonable accuracy.[2] Moreover, these changes can be detected in patients with mild cognitive impairment (MCI) who will progress to AD.[3;4] Brain tissue loss (atrophy) secondary to the neurodegenerative disease process can be visualized and measured using MRI. Whole-brain atrophy rate, measured from serial MRI, correlates well with disease and clinical progression in patients with MCI and AD.[5-7]

Although both MRI and CSF biomarkers have shown to be valuable markers of disease in MCI and AD,[2;8] the relation between these markers has been less well studied. In cross-sectional studies, CSF biomarkers have been reported not to be related to MRI measures of atrophy, suggesting that these markers reflect different aspects of Alzheimer type neuropathology.[9;10] However, longitudinal studies are needed, to clarify the relationship between these markers. The few studies that have reported CSF biomarkers and MRI measures in a longitudinal design, have used relatively small sample sizes, and have shown conflicting results in terms of whether or not these markers are associated.[11-13]

The objective of the present investigation was to assess whether MRI measures and CSF biomarkers are related or provide independent information. We therefore assessed the relationship between baseline levels of CSF $A\beta_{1-42}$, tau, and ptau-181 and whole-brain atrophy rate in patients with AD, MCI, and controls. In addition, we studied the association between longitudinal change of these CSF biomarker levels, whole-brain atrophy rates, and change in cognitive function.

Methods

Patients

We included 47 patients with AD, 30 patients with MCI and 24 controls with baseline CSF and repeat MRI scans from our memory clinic. All patients underwent lumbar puncture (LP) at baseline and MRI at baseline and follow up. At follow-up, 49 patients (20 AD, 18 MCI, 11 controls) agreed to undergo a second lumbar puncture. Follow-up time was defined as time between the two MRI scans (mean interval 1.7 years, standard deviation

0.7; range 11m-4y2m). Patients underwent a standardized clinical assessment including medical history, physical and neurological examination, psychometric evaluation, and brain MRI. The Mini-Mental State Examination (MMSE) was used as a measure of general cognitive function.[14] Diagnoses were established during a multidisciplinary consensus meeting according to the Petersen criteria for MCI[15] and the NINCDS-ADRDA (National Institute of Neurological and Communicative Diseases and Stroke/ Alzheimer's Disease and Related Disorders Association) criteria for probable AD.[16] The team involved in the diagnostic work-up was not aware of the results of the CSF analyses or the whole-brain atrophy rates. The control group consisted of 19 patients who presented to our memory clinic with subjective complaints, but who had normal clinical investigations and did not have significant cognitive deficits (i.e. MCI criteria were not fulfilled). Additionally, we included 5 volunteers without cognitive complaints, who underwent the same diagnostic procedure as patients attending our memory clinic. The study was approved by the institutional ethical review board and all subjects gave written informed consent.

Clinical assessment at follow-up

Non-demented subjects (MCI and controls) visited the memory clinic annually. Diagnostic classification was re-evaluated at follow-up. The clinical diagnosis of dementia was determined according to published consensus criteria.[16;17] Within the MCI group, 12 patients remained stable, 17 progressed to AD,[16] and one to fronto-temporal lobar degeneration (FTLD).[17] Within the control group two progressed to MCI, two to AD and one to FTLD, while 14 controls remained stable.

MRI

MR imaging was performed on a 1.0-T Siemens Magnetom Impact Expert scanner (Siemens AG, Erlangen, Germany) and included coronal T1-weighted 3D MPRAGE volumes (magnetization prepared rapid acquisition gradient echo; single slab 168 slices; matrix 256x256; FOV 250mm; voxel size 1x1x1.5 mm; repetition time=15ms; echo time=7ms; inversion time=300ms; flip angle 15°). All subjects included had two scans of adequate quality, performed on the same scanner using an identical imaging protocol. Scans were reviewed by a radiologist to exclude non-neurodegenerative pathology that could explain the cognitive impairment. Scans that fulfilled radiological criteria of the NINDS-AIREN for vascular dementia were excluded.[18]

Whole-brain atrophy rates were measured with SIENA (Structural Image Evaluation, using Normalisation, of Atrophy), a fully automated technique part of FSL (for a detailed explanation see: http://www.fmrib.ox.ac.uk/analysis/research/siena/).[19]

Briefly, the brain was extracted using the brain extraction tool.[20] Compared to standard SIENA, the procedure to remove non-brain tissue was slightly modified, because the brain extraction tool often leaves significant amounts of non-brain tissue (e.g. skull, meninges), while also removing cortex in some areas.[21] To remove all non-brain tissue without losing cortex, we incorporated in the procedure the registration of a template mask to the individual scans. After this modified brain extraction procedure, the standard SIENA pipeline was continued. Using affine registration, the two scans were resampled in a common space to allow the change analysis. The skull was used as a scaling constraint in this step, in order to prevent the registration from introducing differences in head size between the two time points. The change analysis was then performed by applying automated tissue type segmentation, identifying edge points between brain tissue and other substances, and then estimating the perpendicular motion of the brain edge at these edge points. Finally, the average edge motion was converted to a percentage brain volume change (PBVC) between the two time points. For SIENA, an error of 0.15 to 0.20% on the PBVC scale has been reported.[19] All individual scans, registration results, and SIENA output were reviewed by a rater who was blinded to the diagnosis.

CSF

CSF was obtained by LP between the L3/L4 or L4/L5 intervertebral space, using a 25-gauge needle, and collected in 12-mL polypropylene tubes. Within two hours, CSF samples were centrifuged at 2100*g* for 10 minutes at 4°C. A small amount of CSF was used for routine analysis, including total cells (leucocytes and erythrocytes), total protein and glucose. CSF was aliquoted in polypropylene tubes of 0.5 or 1 ml, and stored at -80°C until further analysis. CSF $A\beta_{1-42}$, tau and ptau-181 were measured by commercially available sandwich ELISAs (Innotest beta-amyloid$_{1-42}$,[22] Innotest hTau-Ag,[23] and Innotest Phosphotau $_{(181P)}$,[24] Innogenetics, Ghent, Belgium). The intra-assay coefficient of variation (CV) was 2.8% for $A\beta_{1-42}$, 3.7% for tau and 1.6% for ptau-181. The inter-assay coefficient of variation (CV) was 13.5% for $A\beta_{1-42}$, 10.2% for tau and 12.8% for ptau-181. To circumvent inter-assay variability, baseline and follow-up samples were run in the same assay at the time of the second spinal tap.[25]

Statistical analysis

Statistical analysis was performed with SPSS 12.0 (2003, Chicago, IL). Whole-brain atrophy rate (PBVC), change in CSF biomarker levels, and change in MMSE over time were annualized by dividing by the time interval in years. A more negative whole-brain atrophy rate represents a larger relative brain volume loss per year. CSF biomarker

levels were log-transformed. Frequency distributions for sex were compared with chi-squared tests. One way Analysis of Variance (ANOVA) with post hoc Bonferroni tests was used to compare continuous variables between the diagnostic groups. To assess associations between baseline CSF biomarker levels and whole-brain atrophy rate, we first calculated Pearson's correlations across the whole group. We then used linear regression analyses with whole-brain atrophy rate as dependent variable, to adjust for age, sex and diagnosis (using dummy variables). Separate models were used for each CSF biomarker. To check if associations with CSF biomarker levels differed according to diagnostic group, interaction terms (dummy-diagnosis * CSF biomarker) were included in the model. If there was a significant interaction between diagnosis and CSF biomarker ($p \leq 0.05$), β[SE] are displayed for each diagnostic group separately. When no significant interaction was found, the overall β is reported. Finally, associations between annualized whole-brain atrophy rate, annualized change in CSF biomarker levels, and annualized change in MMSE score were assessed using bivariate correlations (available for 47 patients).

Results

Demographic and clinical data are presented by patient group in Table 1. MCI patients were older when compared to AD patients. We found no difference in sex or follow-up time. Annualized whole-brain atrophy rate differed between diagnostic groups ($p<0.001$). We also found group differences for baseline $A\beta_{1-42}$ ($p<0.001$), tau ($p=0.001$), and ptau-181 ($p=0.006$). By contrast, annualized change in CSF $A\beta_{1-42,}$ tau, and ptau-181 levels over time did not differ between the patient groups (all $p>0.45$).

To investigate associations between baseline CSF levels of $A\beta_{1-42,}$ tau, and ptau-181 and whole-brain atrophy rate, we first performed bivariate correlations across the whole sample, as shown in Figure 1. Lower baseline CSF levels of $A\beta_{1-42}$ ($r=0.30$, $p<0.01$) and higher tau levels ($r=-0.25$, $p=0.01$) were associated with a higher whole-brain atrophy rate, while CSF ptau-181 levels were not ($r=-0.13$, $p=0.18$). After adjustment for age, sex, and diagnosis in linear regression analyses we found no association between $A\beta_{1-42}$ and whole-brain atrophy rate (β[SE] 0.14[0.25], $p=0.58$). The interaction terms for CSF biomarker and diagnosis were significant for tau ($p=0.02$) and ptau-181 ($p=0.02$), implying that associations of these CSF biomarkers and whole-brain atrophy rate were different for the diagnostic groups. In the control group there was a trend for increased tau to be associated with a higher whole-brain atrophy rate (β[SE] -0.62 [0.32], $p=0.06$), however after exclusion of three patients who progressed to dementia the effect

disappeared. Furthermore, this effect was not observed in MCI (β[SE] -0.30 [0.34], p=0.38), or AD (β[SE] 0.43 [0.27], p=0.12). By contrast, increased ptau-181 levels were associated with a lower whole-brain atrophy rate (β[SE] 0.77 [0.36], p=0.04) in the AD group. The effects in the control group (β[SE] -0.55[0.41], p=0.18) and MCI group (β[SE] -0.23 [0.40], p=0.57), though not significant, were in the opposite direction of that in the AD group.

Table 1. *Demographics and clinical variables by diagnostic group*

	Control n=24	MCI n=30	AD n=47
Age-at-diagnosis (y)	66 (9)	70 (6)	65 (8) [b]
Sex (f/m)	11 / 13	16 / 14	25 / 22
Baseline MMSE score	29 (2)	26 (3) [a]	22 (5) [d,e]
Annualized change in MMSE score *	-0.1 (1.1)	-1.5 (2.7) [a]	-2.2 (1.8) [d]
Diagnosis at follow up	2 MCI 2 AD 1 FTLD	17 AD 1 FTLD	-
Follow-up time (y)	1.7 (1.0)	1.9 (0.6)	1.7 (0.5)
Annualized whole-brain atrophy rate (%/y)	-0.6 (0.6)	-1.2 (1.1) [a]	-1.9 (1.0) [d]
Baseline $A\beta_{1-42}$	713 (258)	503 (232) [d]	384 (119) [b,d]
Baseline tau	459 (382)	583 (283)	819 (463) [b,c]
Baseline ptau-181	63 (33)	79 (33)	91 (34) [c]
Annualized change in CSF $A\beta_{1-42}$ level [+]	37 (74)	16 (34)	32 (25)
Annualized change in CSF tau level [+]	23 (37)	30 (45)	65 (46)
Annualized change in CSF ptau-181 level [+]	1 (2)	2 (4)	0.2 (5)

Data are presented as mean (sd), unless indicated otherwise. Differences between groups were assessed using ANOVA with age and sex as covariates and post-hoc Bonferroni correction. Please note that raw values are shown for CSF biomarkers (pg/mL), while log-transformed variables were used for statistical analysis.
MCI=mild cognitive impairment;AD=Alzheimer's disease; FTLD=Frontotemporal Lobar Degeneration; MMSE=mini-mental state examination; CSF=Cerebrospinal fluid; $A\beta_{1-42}$=beta-amyloid$_{1-42}$; ptau-181= tau phosphorylated at threonine-181; MRI=Magnetic Resonance Imaging.
[a] $p<0.05$ compared to controls; [b] $p<0.05$ compared to MCI; [c] $p<0.01$ compared to controls; [d] $p<0.001$ compared to controls; [e] $p<0.001$ compared to MCI
[+]=available for n=49 ($A\beta_{1-42}$, tau) and n=47 (ptau-181)
*=available for n=93

Finally, we studied associations between change in CSF biomarker levels over time, and whole-brain atrophy rate. Across groups, change in $A\beta_{1-42}$ (r=-0.01, p=0.93), tau (r=-0.10, p=0.49), and ptau-181 (r=-0.11, p=0.48) levels were not associated with whole-brain atrophy rate. In addition, we assessed longitudinal associations of change in CSF biomarker levels, whole-brain atrophy rate, and change in MMSE score over time.

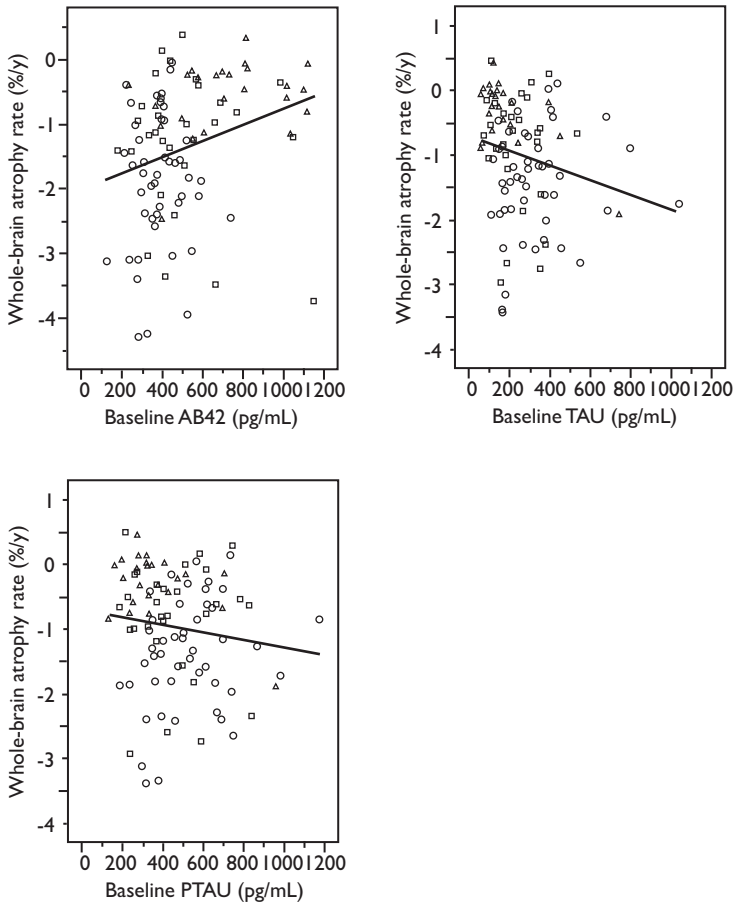

Figure 1. *Scatter plots of baseline CSF biomarker levels by annualized whole-brain atrophy rate. (**A**) Across diagnostic groups baseline Aβ1-42 levels and whole-brain atrophy rate were associated (r=0.30, p<0.01). In diagnostic groups no association was found. (**B**) Across diagnostic groups tau levels and whole-brain atrophy rate were associated (r=-0.25, p=0.01). In the control group there was a trend for increased tau to be associated with a higher whole-brain atrophy rate (β[SE] -0.62 [0.32], p=0.06). However, this effect disappeared when three patients, who progressed to dementia, were excluded. (**C**) Across diagnostic groups ptau-181 levels were not associated (r=-0.13, p=0.18). By contrast, there was a modest effect of an increased ptau-181 level in the AD group (β[SE] 0.77 [0.36], p=0.04) being associated with a lower whole-brain atrophy rate.*

Please note that raw CSF values are shown, while statistical analyses were performed using log-transformed values. —=fit line across groups Δ=controls; □=MCI; ○=AD

While whole-brain atrophy rate was associated with change in MMSE score (r=0.40, p<0.05), change in CSF levels of $A\beta_{1-42,}$ (r=0.18, p=0.22), tau (r=-0.03, p=0.83), and ptau-181 (r=-0.01, p=0.97) were not.

Discussion

The major finding of this study is that, notwithstanding modest correlations of baseline CSF biomarker levels and whole-brain atrophy rate across groups, hardly any association within diagnostic groups was found. Whole-brain atrophy rate was associated with clinical progression, measured by change in MMSE score, but longitudinal changes in the CSF biomarker levels were not. Thus, MRI and CSF biomarkers appear to reflect different aspects of AD: whole-brain atrophy rate appears to be linked to the clinical progression of the disease, whereas CSF biomarkers seem to reflect disease state rather than rate of progression.

Both CSF biomarker levels and atrophy on MRI are used in the diagnostic work-up of AD.[2;7;8] Moreover, both marker types are predictive of dementia in patients with MCI.[3,4,26,27] Previous studies typically report lowered CSF levels of $A\beta_{1-42}$, and elevated tau and ptau, and higher rates of whole brain atrophy in MCI and AD.[28;29] Our study confirms these results, which have been published previously in overlapping samples, derived from the same memory clinic population.[30;31] Relatively few studies have combined CSF biomarker levels and atrophy measured f rom MRI, using a cross-sectional design[3;9;10] or a longitudinal design.[11-13] Of the longitudinal studies one study described positive correlations between baseline CSF biomarkers and change in MRI measures in a group with a wide variation in cognitive impairment.[11] A second study described the relation between increase in tau phosphorylated at threonine-231 (ptau-231) and $A\beta_{1-42}$ and decrease in hippocampal volume in seven patients with MCI.[12] Finally, a study involving 22 AD patients found high baseline CSF levels of ptau-231 to be associated with a higher rate of hippocampal atrophy.[13] In the present study, however, we were not able to confirm these findings despite our larger patient sample.

For tau we found an association *across* groups with, as expected, higher levels of tau being related to a faster rate of atrophy; however we did not find this association *within* diagnostic groups. A trend towards higher tau being associated with higher whole-brain atrophy rates within the control group, could be ascribed to a few subjects showing clinical progression, since the effect disappeared after exclusion of three subjects who progressed to dementia. It might be argued that these subjects should not have been included in the control group. However, because the risk of dementia increases with

age, healthy elderly may progress to dementia.[32] Moreover, the cognitive continuum of dementia shows a gradual decline, and boundaries between AD and MCI are somewhat arbitrary.[33] We therefore think by including these progressing patients, we included the whole cognitive spectrum and studied a typical heterogeneous memory clinic population.

In contrast to $A\beta_{1-42}$ and tau, baseline ptau-181 was weakly associated with whole-brain atrophy rate within the AD group, but not across groups. When we started this study, we hypothesised that patients with a larger load of senile plaques and neurofibrillary tangles (reflected by CSF biomarker levels), would have a higher rate of neuronal loss, consequently leading to a higher whole-brain atrophy rate. Our study did not confirm this. In fact, we found a modest effect in the opposite direction, with a higher (more abnormal) ptau-181 being related to a lower (less abnormal) whole-brain atrophy rate. We are unsure how to interpret this finding. We cannot exclude the possibility that some of our AD patients were misdiagnosed, especially since no post mortem verification of diagnosis was available. However, all patients fulfilled NINDS-ADRDA clinical criteria for probable AD, which was confirmed both at baseline and at follow-up in multidisciplinary consensus meetings.

Post mortem studies have shown considerable overlap in the neuropathological features associated with AD, regardless of whether or not dementia was actually present during life.[34] This implies that other factors than senile plaques and neurofibrillary tangles must be involved in the development of the clinical syndrome of dementia. Indeed, it has been reported that brain volume by itself is a good predictor of dementia, independent of senile plaque and neurofibrillary tangle load.[34] Our results are in line with these neuropathological findings, since we found hardly any association of whole-brain atrophy rates and CSF biomarker levels. This could imply that brain volume loss in vivo, measured with MRI, and CSF biomarker levels, which are thought to represent senile plaque and neurofibrillary tangle load, reflect different aspects of AD.

Among the strengths of this study is that we investigated the association of two widely used markers (CSF and MRI) in a large cohort of MCI, AD patients and controls derived from a memory clinic, in a prospective longitudinal fashion. For every patient, baseline CSF and longitudinal MRI were available. Follow up CSF data were available for a large subgroup. A limitation of this study may be that we used MRI scans that were obtained on a 1T scanner. We feel however that T1 scans have sufficient contrast of parenchyma-CSF, while the gain of scans obtained at a higher field strenght largely lies in increased gray-white matter contrast. As we assessed the whole-brain, rather than gray and white matter separately, we feel that our scans had sufficient quality. In addition, it could be argued that hippocampal atrophy is a more specific marker

for AD than whole-brain atrophy rate,[35] which is increased in a number of different diseases that cause dementia.[36;37] However, senile plaques and neurofibrillary tangles accumulate throughout the brain, and are not exclusively found in the medial temporal lobe.[38]

In contrast to whole-brain atrophy rates which were associated with change in MMSE score over time, longitudinal changes in CSF biomarker levels were not. These results suggest that for tracking the rate of progression of AD, whole-brain atrophy rates are more useful than CSF levels of $A\beta_{1-42}$, tau, and ptau-181; by contrast these CSF markers can be considered to be disease state markers, which may be more sensitive as diagnostic tools, possibly in earlier stages of AD.

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New research criteria for the diagnosis of Alzheimer's disease applied in a memory clinic population

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Submitted

Abstract

Objective: To apply newly proposed research criteria for Alzheimer's disease in a memory clinic population using clinical criteria as golden standard.

Methods: 138 AD patients, 145 non demented subjects and 78 patients with other dementias were included. Dichotomized medial temporal lobe atrophy (MTA) score on MRI and dichotomized CSF profiles (based on beta-Amyloid$_{1-42}$, tau and phosphorylated tau at threonine-181 levels) were used in combination with an episodic memory test to assess sensitivity and specificity of the newly proposed criteria and their components separately.

Results: We found a specificity of 96% for comparison with non demented subjects and 50% compared with other demented patients with a sensitivity of 86% for AD. Specificity was highest (100% and 78%) when both MTA score and CSF profile were abnormal in addition to the episodic memory test (sensitivity 43%).

Conclusion: The newly proposed research criteria for AD yield a good specificity for comparison with non demented subjects. When there is clinical doubt, however, about the type of dementia, at least two supportive features should be considered (i.e. abnormal MTA score and CSF profile) in addition to memory impairment as core diagnostic criterion.

Introduction

Alzheimer's disease (AD) is the most common form of dementia. The clinical criteria that are commonly used for the diagnosis of probable AD, were proposed more than 20 years ago and largely depend on exclusion of other dementias.[1] Even in patients who have been followed up clinically for several years at expert research centres, the diagnostic accuracy is relatively low, with specificity of around 70% and sensitivity of 80%.[2] A definite diagnosis of AD can only be made at autopsy.

The major histopathological hallmarks of AD are senile plaques, containing beta-Amyloid$_{1-42}$ (Aβ_{1-42}) and neurofibrillary tangles with microtubuli-associated tau protein. These pathological changes start in the medial temporal lobe and are present before the onset of clinical dementia.[3;4] Research in the past decade has focussed on identification of Alzheimer pathology using biomarkers. On MRI, atrophy of the medial temporal lobe is a marker of AD.[5] In cerebrospinal fluid (CSF), Aβ_{1-42} levels and elevated (phosphorylated) tau protein levels can differentiate patients with AD from control subjects or patients with other neurological conditions with relatively high accuracy.[6]

In planning of trials with disease-modifying treatments, especially in the earliest stages of the disease, it is of the uttermost importance to include only those patients who are most certain to actually have AD. This is not only essential to maximize the chance of successful treatment but also to limit the exposure of potentially toxic therapies to those with AD. More importantly, those who have non-AD dementia or no dementia at all should be reliably excluded. In a recently published position paper new research criteria for the diagnosis of AD were proposed that would allow diagnosis related to neuropathological changes in AD, allowing intervention at an early or preclinical stage.[7] The authors suggest to make use of supportive features on MRI, CSF and FDG-PET, that identify the AD-associated structural and molecular changes in the brain and their biochemical footprints, in combination with the core diagnostic criterion of episodic memory impairment.

In this study we applied the above mentioned new research criteria, using MRI and CSF in addition to memory function, in our memory clinic population. FDG-PET was not routinely available. We aimed to compare the presently widely used clinical criteria according to the NINCDS-ADRDA with the newly proposed criteria in AD patients versus non demented controls and versus other dementias.

Methods

Study population

361 patients were recruited at the Alzheimer Center of the VU Medical Center (VUMC) between October 2000 and April 2007. The group comprised 138 AD patients according to the NINCDS-ADRDA criteria[1], 78 patients with other dementias (43 frontotemporal lobar degeneration (FTLD) according to the criteria of Neary and Snowden for FTLD[8], 7 vascular dementia (VaD) according to the NINDS-AIREN criteria for VaD[9], 15 dementia with Lewy bodies (DLB) according to the criteria of McKeith et al for DLB[10], 4 corticobasal degeneration and 3 progressive supranuclear palsy according to previously published criteria[11-13], 1 Huntington disease and 1 hereditary Creutzfeldt Jacob disease confirmed genetically and 4 with undetermined aetiology of dementia) and 145 subjects without dementia (90 patients with subjective complaints, 55 other neurological or psychiatric disorders). Patients underwent a standardized clinical assessment, including medical history, physical and neurological examination, psychometric evaluation, laboratory tests, lumbar puncture (LP), EEG and brain MRI. The diagnoses were made by a multidisciplinary team of neurologists, neuropsychologists, a neurophysiologist, a psychiatrist, a geriatrician and a radiologist. Psychometric evaluation, MRI and LP were performed within one year of the baseline diagnosis. The study was approved by the ethical review board of the VUMC and all subjects gave written informed consent.

Neuropsychological assessment

The neuropsychological test battery was designed to screen the major cognitive functions and included the Mini-Mental State Examination (MMSE) and the Visual Association Test (VAT)[14;15]. The MMSE was used to assess dementia severity. For assessment of episodic memory the VAT was used. Patients are shown cue cards with an object and association cards with the previously seen object plus an interacting object and are asked to name each object. Subsequently, the cue cards are shown and patients are asked to recall the now missing interacting objects (score 0-12). The memory scores were dichotomized, with a score of 11 or 12 considered normal, and a score below 11 considered abnormal.

CSF analysis

CSF was obtained by LP between the L3/L4 or L4/L5 intervertebral space, using a 25-gauge needle, and collected in 12-mL polypropylene tubes. Within two hours, CSF samples were centrifuged at 2100*g* for 10 minutes at 4°C. A small amount of CSF

was used for routine analysis, including total cells (leucocytes and erythrocytes), total protein and glucose. CSF was aliquoted in polypropylene tubes of 0.5 or 1 ml, and stored at -80°C until further analysis. CSF $A\beta_{1-42}$, tau and tau phosphorylated at threonine-181 (ptau-181) were measured by commercially available sandwich ELISAs (Innotest beta-Amyloid$_{1-42}$, Innotest hTau-Ag and Innotest Phosphotau $_{(181P)}$, Innogenetics, Ghent, Belgium). The inter-assay coefficient of variation for $A\beta_{1-42}$ was 14%, 12% for tau and 9% for ptau-181. The optimal cut off values for CSF $A\beta_{1-42}$, tau and ptau-181 levels were set at data obtained in earlier studies, in which we applied a sensitivity of ≥ 85% for each individual biomarker in accordance with the Ronald and Nancy Reagan Consensus report after drawing Receiver Operating Characteristics curves.[16] The following cut off values were used: CSF $A\beta_{1-42}$ < 495 pg/mL, CSF tau > 356 pg/mL and ptau-181 > 54 pg/mL.[17;18] A CSF profile combining $A\beta_{1-42,}$ tau and ptau-181 was constructed, which was abnormal when at least two of the three biomarkers were abnormal. The team involved in the diagnostic work-up was not aware of the results of the CSF analyses.

MRI analysis

MRI scans were made on a 1.0 Tesla scanner (Siemens Magnetom Impact Expert, Erlangen, Germany) and included a coronal T1-weighted 3D inversion-prepared gradient echo-sequence (168 slices, FOV 250mm, matrix 256x256; slice thickness 1.5 mm, TE: 7ms, TR: 15 ms, TI 300 ms, flip angle 15 degrees). MTA was rated visually using a scale ranging from 0 (no atrophy) to 4 (severe atrophy).[5] MTA scores of the left and right hippocampi were averaged.[4] In one patient only the left MTA score was available because right inferior temporal lobe was compressed by an epidermoidal cyst. Although brain MRI contributed to the diagnostic process by excluding other neurological diseases (e.g. brain tumor), MTA scores were not used in the diagnostic process. Two trained raters, who were blinded to clinical information, rated MTA. To determine reliability, 20 scans were scored twice (weighted Cohen's kappa for intra rater reliability >0.85 and inter rater variability >0.80). MTA scores were dichotomized, and an average MTA ≥1.5 was considered abnormal (requiring a score of at least 2 on one side).

Operationalization criteria

The new research criteria[7] for AD were applied as follows. Patients were defined as having AD according to the new research criteria when there was evidence of episodic memory impairment based on an abnormal VAT-score and additionally positive evidence for the presence of Alzheimer pathology, as evident from abnormal MTA-score AND/OR abnormal CSF profile. To assess the components of the new research criteria separately, sensitivity and specificity of VAT alone, VAT combined with MTA, VAT

combined with CSF and abnormal VAT with abnormal MTA AND CSF profile were additionally determined.

Statistical analysis

For statistical analysis, SPSS version 14.0 (Chicago IL) was used. One way Analysis of Variance (ANOVA) with post hoc Bonferroni tests was used to compare continuous variables between diagnostic groups. Frequency distributions for sex were compared with chi-squared tests. Sensitivities and specificities were calculated for AD versus other dementias, AD versus non-demented controls and AD versus other or no dementia (previous groups combined) using the diagnoses made in multidisciplinary setting according to clinical criteria as golden standard.

Results

The demographic characteristics of the diagnostic groups are presented in table 1. No differences were found for sex. The no dementia group was younger than the AD and other dementias group. According to the baseline MMSE AD and other demented patients were moderately demented. There were group differences for memory score, MTA-score and CSF profile, AD patients having most abnormal scores, the no dementia group having normal scores and the other dementia group scoring mostly intermediate (see table 1 for details).

The specificity and sensitivity of the new research AD criteria are presented in table 2. The new criteria had a good sensitivity (86%), and almost perfect specificity for AD compared to no dementia (96%). When compared with patients with other dementias, specificity was modest (50%). Subsequently, sensitivities and specificities are specified for components of the new criteria. There is a good specificity (86%) and sensitivity (93%) of the memory score alone compared with controls, but as memory impairment is a characteristic of most types of dementia, specificity (33%) was poor in comparison with other demented patients. The specificity for both comparisons increases when either MTA score (99%) or CSF profile (97%) are added to the memory score. This is at the expense of the sensitivity, however, which decreases most for memory score combined with MTA score (49%) and less for memory score combined with CSF profile (80%). Finally, when memory score, MTA score and CSF profile were all abnormal specificity for both comparisons (i.e. versus other and non demented patients) was best (100%), while the sensitivity for AD remained modest (43%).

Table 1. *Demographics of memory clinic population*

	AD	Other dementias	No dementia	p-value
Number	138	78	145	
Sex F/M	70/68	27/51	67/78	0.15
Age (years)	68 (8)	66 (9)	60 (10)	< 0.01 [a,b]
MMSE#	21.5 (4.6)	22.7 (5.7)	28.3 (1.8)	< 0.01 [c,d]
Memory-score	4.7 (3.5)	7.5 (4.0)	11.4 (1.3)	< 0.01 [c,d,e]
MTA-score	1.4 (0.9)	1.4 (1.1)	0.3 (0.5)	< 0.01 [a,b]
$A\beta_{1-42}$ (pg/ml)	443 (171)	706 (294)	817 (223)	< 0.01 [c,d,e]
Total Tau (pg/ml)	768 (466)	458 (359)	300 (206)	< 0.01 [a,b,f]
Ptau-181 (pg/ml)	89 (39)	57 (28)	48 (22)	< 0.01 [a,f]

Data are presented as mean (standard deviation). AD=Alzheimer's disease, MCI=mild cognitive impairment, $A\beta_{1-42}$=beta-Amyloid$_{1-42}$, ptau-181=phosphorylated tau at threonine-181, MTA=mean medial temporal lobe atrophy score.
Memory score based on visual association test.[15]
Baseline MMSE was missing for 1 non-demented subject, 2 AD patients and 5 other demented patients.
[a] no dementia <AD, [b] no dementia < other dementias, [c] no dementia >AD,
[d] no dementia > other dementias, [e] other dementias >AD, [f] other dementias <AD

Table 2. *Specificity of revised AD criteria in a memory clinic population*

	AD versus no dementia (n=145)	AD versus other dementia (n=78)	AD versus other or no dementia (n=223)	Sensitivity (n=138 AD patients)
New criteria (memory + MTA OR CSF)	96%	50%	80%	86%
Memory	86%	33%	68%	93%
Memory + MTA	99%	60%	86%	49%
Memory + CSF	97%	67%	86%	80%
Memory + MTA AND CSF	100%	78%	92%	43%

AD=Alzheimer's disease. MTA=medial temporal lobe atrophy.
Memory = visual association test score[15] abnormal < 10, MTA-score abnormal ≥1.5, CSF abnormal when two of the three biomarkers abnormal (cut off values: $A\beta_{1-42}$ <495, tau >356, ptau-181 >54 pg/mL).
Note that specificity represents the percentage of other or non demented subjects with normal test scores and sensitivity the percentage of AD patients with abnormal test scores. Thus another control group (i.e. no or other dementia) does not change the sensitivity.

Discussion

This study aimed to compare the newly proposed research criteria for AD with clinical diagnoses in a memory clinic population. We found an almost perfect specificity of 96% for comparison with non demented subjects and a modest specificity of 50% compared with other demented patients with a good sensitivity of 86% for AD patients.

The specificity represents the percentage of patients without AD and normal test scores. The criteria stress the importance of a good specificity, in order to exclude patients with non AD dementia and limit the exposure of possibly toxic therapies to those who have pure AD. Sensitivity represents the percentage of AD patients who have abnormal test scores. Pure AD patients are most likely to benefit from anti-Alzheimer medication, directed against AD neuropathology. The specificity in comparison with non demented subjects is almost perfect, which supports use of these criteria for selection of therapeutic trials with anti-Alzheimer medication. The specificity for other dementias, however, is modest. This is in agreement with previous studies, showing overlap of memory dysfunction, abnormal MTA-score and CSF profile between patients with AD and patients with other dementias.[6;19;20] When in addition to memory function either MTA score or CSF profile are abnormal specificity increases (from 33% to 60% and 67%), but when both MTA score and CSF profile are abnormal in addition to memory function, specificity in comparison with other dementias reaches 78%. Thus, in case of clinical doubt about the type of dementia, a combination of memory impairment as core diagnostic criterion and at least two supportive features should be considered, instead of one supportive feature that is presently proposed in the new criteria. This accounts especially for trials with anti-Alzheimer medication.

Memory score in combination with MTA score yielded a higher specificity in the comparison with non demented subjects than the combination of memory score and CSF profile. In the comparison with other dementias, however, memory score combined with CSF profile yielded a higher specificity. Moreover, the combination of memory score and CSF profile yielded a higher sensitivity. This may suggest that CSF biomarkers are more specifically related to AD pathology than hippocampal atrophy. This corresponds with results from earlier studies, suggesting variable causes of hippocampal atrophy. E.g. in FTLD hippocampal atrophy may be present, but based on other neuropathological changes than plaques and tangles found at autopsy.[21] Thus, causes of an abnormal MTA score may be diverse, while abnormal CSF biomarkers are thought to reflect AD pathology only.[6;19]

One might argue, that patients with other dementias (e.g. FTLD, VaD and DLB) clinically, do not have AD and that it seems artificial to subject them to criteria for AD.

These patients fulfil previously published clinical criteria that could be used to exclude these patients from this study. On the other hand, in clinical practice, the difference between patients with AD and FTLD, VaD or DLB it is not always obvious. Moreover, patients with these dementia types often have additional AD pathology at autopsy. Thus these patients might benefit from anti amyloid therapy as well. We feel that, that the application of the newly proposed criteria to various other dementias gives a good insight in the usability of these criteria in a memory clinic population and may help in selecting patients that might benefit from anti-Alzheimer medication.

A possible limitation in our study is our measure of memory impairment. We used the visual association test (VAT), which provides a measure of immediate memory. According to the new research criteria, a delayed recall test should be used to assess early episodic memory impairment related to AD. Unfortunately we do not have sufficient delayed recall data for our memory clinic population. This may have influenced the results, especially in early AD, when memory impairment is in the foreground. The new research criteria also mention, however, that (early) AD patients show reduced benefit from cueing at recall, which should be used for measuring memory impairment. VAT is a specifically cued task. It has been demonstrated that the VAT with testing associative learning, is highly specific and sensitive for detecting (early) AD and excluding non-AD subjects.[15] Therefore, we feel that the VAT is a strong alternative for measuring memory impairment using a delayed recall task.

Another limitation is the fact that MRI was part of the diagnostic work up of our memory clinic population, e.g. to exclude other neurological diseases like brain tumor.[2] Using MRI during the diagnostic process, may have slightly influenced our results, as the presence of MTA may unconsciously have influenced the physicians to a diagnosis of AD. We therefore cannot entirely exclude the possibility of circular reasoning resulting in an overestimation for the specificity and sensitivity of the MTA-score.

Another limitation is the lack of FDG-PET scan data in our memory clinic population. A reduction of glucose metabolism as seen on PET in bilateral temporal parietal regions and in the posterior cingulate is a commonly described diagnostic criterion for AD.[22] Unfortunately FDG-PET was only rarely used in our memory clinic population, thus we chose to make use of MRI and CSF only. Both MTA on MRI and CSF biomarkers have been shown to discriminate AD from controls and other dementias with comparable accuracy as FDG-PET. [22] Moreover, in general they are more easily available and less time consuming in clinical practice than PET scans.

Another limitation of this study is the lack of neuropathological confirmation of our patient population. Since definite diagnoses of AD and other dementias can only be made at autopsy, this would indeed help substantially to get a deeper understanding

and true reproduction of the specificity and sensitivity of the newly proposed criteria. Moreover, the anti-Alzheimer therapies that are being developed are based on the specific neuropathological changes in AD. We feel that, although we lack FDG-PET scan and neuropathological data, this study gives a good insight into the categorization of a memory clinic population after applying the newly proposed criteria. For an optimal selection of patients that would benefit from specific anti-Alzheimer therapies, future studies with neuropathological diagnoses are needed assessing the sensitivity and specificity of the newly proposed criteria.

In conclusion, the newly proposed AD criteria yield a high specificity in subjects with no dementia. When there is clinical doubt about the type of dementia, two (instead of the presently proposed one) supportive feature should be abnormal in addition to the core criterion of memory impairment, especially in case of therapeutic trials with anti-Alzheimer therapies. Preferably neuropathological confirmation of AD, however, is needed to assess the usability of the newly proposed criteria for application of specific anti-Alzheimer therapies. In future, PIB-PET scans may possibly render these criteria redundant, for administration of amyloid plaque specific therapies.

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Chapter 6

Summary
General discussion
Recommendations

Summary

The general objective of this thesis was to explore longitudinal aspects of CSF biomarkers and post mortem changes of these CSF biomarkers. In addition the value of CSF and MRI was compared for discriminating (incipient) AD in memory clinic patients. First, we found a higher variability of baseline and follow-up $A\beta_{1-42}$ levels when assessed in different assays compared to assessment in the same assay. Therefore, in case of repeated spinal taps, determination of $A\beta_{1-42}$ levels should be performed in the same assay. Subsequently we described that the natural course of changes in CSF $A\beta_{1-42}$, total tau and ptau-181 levels over time was comparable in patients with subjective complaints, MCI and AD patients. The cross-sectional difference between groups, however, exceeded by far the longitudinal changes within groups. Thus, repeated assessment of these biomarkers is not useful in a clinical setting, as they are insensitive to disease progression. Second, we found that young and old AD patients have similar CSF biomarker levels, while elderly controls have lower $A\beta_{1-42}$ and higher (p)tau compared to young controls. This may suggest preclinical AD pathology in older controls, which should be borne in mind when using CSF biomarkers in clinical practice. In addition, although young and old AD patients may have similar levels in absolute terms, compared to their control group, the young AD patients *relatively* have more pathological levels, suggesting differences in AD pathology between young and old patients. Third, we concluded that determination of $A\beta_{1-42}$, total tau and ptau-181 levels in post mortem CSF is not useful as (p)tau levels were extremely high and $A\beta_{1-42}$ levels extremely low without differences between AD and LBD patients and controls. Fourth, combining CSF and MRI, we found hardly any association within diagnostic groups for CSF biomarker levels and whole-brain atrophy rate across controls, MCI and AD patients, although there are modest correlations of baseline CSF levels and whole-brain atrophy rate. Whole-brain atrophy rate was associated with clinical progression, measured by change in MMSE score, but longitudinal changes in the CSF biomarker levels were not. In addition we showed that abnormal levels of CSF $A\beta_{1-42}$ and tau and an abnormal MTA-score are associated with a higher risk of progression to AD in MCI patients. The predictive values of CSF biomarkers exceeded that of the MTA-score. MCI patients with both abnormal CSF profile and an abnormal MTA-score, were at an even higher risk to progress to AD. This corresponds with what we found applying the newly proposed research criteria for AD: the combination of both abnormal CSF profile and abnormal MTA score in addition to an abnormal memory score yielded a specificity of 100% (sensitivity 43%), while abnormal VAT score with either abnormal MTA score or abnormal CSF profile yielded a specificity of 96% (sensitivity 86%). We conclude that

MRI and CSF biomarkers appear to reflect different aspects of AD: atrophy measures on MRI appear to be linked to the clinical progression of the disease, whereas CSF biomarkers seem to reflect disease state rather than rate of progression.

General Discussion

1. Longitudinal aspects of CSF biomarkers

Little is known about the methodological issues of assessing longitudinal changes in CSF biomarkers. A meta-analysis demonstrated considerable variability in absolute concentrations of $A\beta_{1-42}$ among centres, even when using the same commercial assay.[1] And a recent study demonstrated the influence of repeated freeze/thaw cycles and storage temperature on the concentration of $A\beta_{1-42}$.[2] Especially when studying longitudinal changes of CSF biomarkers to evaluate disease progression or treatment effects, sample stability and assay variability are important issues. Since ELISA's for measuring $A\beta_{1-42}$ levels have been available for more than ten years, it is striking that there are only few studies of changes of $A\beta_{1-42}$ levels over time.[3-7] Remarkable in all these longitudinal studies is that only a few mention intra and inter-assay variability and no study explicitly reports that baseline and follow up CSF samples were assessed in the same assay. All above mentioned studies report wide ranges and/or standard deviations of $A\beta_{1-42}$ levels, corresponding with our finding of large variances. After evaluating changes in CSF $A\beta_{1-42}$ level with time in patients with various cognitive and neurological disorders, we found that the variance of the difference of baseline and follow-up CSF-samples in the same assay was smaller than the variance of the difference of repeated $A\beta_{1-42}$ assessment of the baseline CSF sample in different assays. This suggests that, even with acceptable within and between assay variation as judged from the results of the quality control pools, the biological change over time within subjects is smaller than the measuring error. The ultimate implication of this finding may be, that with the methodological limitations of the present ELISA, repeated $A\beta_{1-42}$ determination is not useful in a clinical setting. In addition, repeated $A\beta_{1-42}$ assessment in clinical trials as biomarker of progression in AD may be premature at this stage.

So far, only small studies (sample sizes between 19 and 53) described longitudinal changes of $A\beta_{1-42}$, tau or ptau levels in CSF mainly in AD, while most studies assessed one ore two biomarkers only.[3;4;6-11] The majority showed no changes of these CSF biomarkers over time. One study showed that $A\beta_{1-42}$ levels decrease over time,[7] a second study described an increase of tau level with stable $A\beta_{1-42}$ level,[6] and a third study showed stable $A\beta_{1-42}$ and tau levels but an increase of ptau over time.[9] Few

small studies described longitudinal levels of CSF biomarkers in MCI patients,[4;8] while especially this group of patients is of interest for early therapeutic intervention. This thesis describes longitudinal measurements of CSF biomarkers in patients with subjective complaints, MCI and AD and showed that CSF $A\beta_{1-42}$ and tau, but not ptau-181 levels increased over time, with comparable change in all diagnostic groups. Remarkably, the cross-sectional difference between groups, exceeded by far the longitudinal changes within groups.

Our study is relatively unique in including patients with subjective complaints who underwent two lumbar punctures. Inclusion of healthy controls is preferred, though, because patients with subjective complaints performing well in neuropsychological tests have an increased risk for future cognitive decline.[12] According to a recent study on diurnal fluctuation of $A\beta_{1-42}$ levels, these controls should all be punctured at the same time of day.[13] There are ethical issues, however, for obtaining CSF in healthy controls, especially when subjects have to undergo two lumbar punctures. Also, the discussion of "what is an appropriate control", is a difficult one, with more than one possible answer. For example older healthy controls are at increased risk of dementia, as age is the most important risk factor for dementia.[14] Moreover, studies of CSF biomarkers with a large number of controls are limited, especially controls that underwent more than one lumbar puncture. Thus, although the subjective complaints group is relatively small, and our conclusions need some caution in this respect, inclusion of this patient group also is one of the strengths of the study.

In conclusion, repeated assessment of CSF $A\beta_{1-42}$, tau and ptau-181 is currently not useful in a clinical setting, as they are insensitive to disease progression. Should it nevertheless be deemed necessary to perform determination of $A\beta_{1-42}$ levels in multiple specimens of the same patient, we advise to run these in the same assay.

2. CSF biomarkers reflecting neuropathology

In agreement with the criteria of the Consensus Report of the Working Group on Molecular and Biochemical Markers of AD for an ideal biomarker,[15] CSF biomarkers are thought to reflect neuropathology. Post mortem studies have shown that in young patients with AD there is a strong correlation between dementia severity and plaque and tangle burden, whereas this association is not found in elderly patients.[16] Furthermore, plaque and tangle load as well as the cholinergic deficits may be more severe in young than in old AD patients.[17;18] Therefore, one would expect differences in CSF biomarker levels for young and old AD patients. Few studies have analysed differences in CSF biomarker levels in patients with early versus late onset AD and came up with inconsistent results.[3;19-22] This thesis describes that the difference in CSF

biomarker levels between young AD patients and controls is larger than the difference between old AD patients and controls. As CSF biomarker levels between young and old AD patients were similar, this was attributable to the old control group having lower $A\beta_{1-42}$ and higher (p)tau compared to the young control group. This suggests that the observed values in elderly controls represent underlying, clinically silent AD pathology. This may imply that age-dependent reference values should be used when applying CSF analysis in clinical practice. On the other hand, there is more overlap in CSF biomarker levels at older age between controls and patients.[23;24] This may favour the use of one set of reference values irrespective of age in combination with careful follow up of older subjects with abnormal CSF biomarkers. In addition, these findings support adding CSF analyses in the diagnostic work up of young patients with cognitive dysfunction.

A possible limitation is the fact that the control group for a large part comprised patients with subjective complaints and the lack of neuropathological information about this group. As discussed in the previous chapter, these patients are known to have a higher risk of developing AD in the future.[12;25;26] However, patients with subjective complaints were equally distributed over the young and old control group. This suggests that the more pathological levels in the old control group are not (only) explained by the higher risk of pre-clinical AD in patients with subjective complaints, but more so by the fact that older individuals in general are more prone to have amyloid plaques with neurofibrillar changes and are at higher risk of developing AD.[27] These findings are congruent with former studies, in which an increased ratio of CSF tau / $A\beta_{1-42}$ or reduced CSF $A\beta_{1-42}$ level has been described to predict cognitive decline in nondemented older adults, suggesting that CSF biomarkers can detect the underlying disease process even during the pre-clinical stage.[28-31] Moreover, previous post mortem and animal studies, described an increase of plaques and tangles and beta-Amyloid with age in non demented individuals and wild type mice.[27;32;33]

We hypothesized that, in contrast to intra vitam CSF, post-mortem CSF might yield additional information reflecting neuropathology, especially when accompanied by the definite diagnosis made at autopsy. Few studies analysed CSF biomarkers for AD in post mortem CSF showing no differences between AD and controls.[34;35] This thesis describes post mortem CSF biomarker levels of $A\beta_{1-42}$, tau and ptau-181 in AD and Lewy body dementia (LBD) patients versus controls. Intra vitam, these biomarkers are known to differentiate AD from controls or Parkinson's disease (PD) with reasonable accuracy.[36-38] In addition, ptau-181 has been suggested to discriminate AD from dementia with Lewy bodies (DLB) and PD from Parkinson's disease with dementia (PDD), although overlap occurs between groups, being consistent with the clinical

overlap between AD and DLB and the neuropathological overlap between PD, PDD and DLB.[39-41]

In our post mortem CSF study we were unable to discriminate patients from controls as $A\beta_{1-42}$ levels were immeasurably low and tau and ptau-181 levels were immeasurably high in all diagnostic groups.

Tau and ptau-181 are neuronal proteins located within the cell. The extremely high levels of tau and ptau-181 are very likely to be explained by massive neuronal cell death, followed by release of intracellular proteins. This finding corresponds with previous post mortem CSF studies showing elevated levels of intracellular proteins released due to necrosis of the brain after death and it is corroborated by the correlation of time between death and lumbar puncture and both tau and ptau-181 levels we found.[42;43] It is also in accordance with previous findings of high (p)tau levels in Creutzfeldt Jakob disease, stroke and trauma reflecting considerable neuronal cell death in a short time period.[44-46] In contrast to (p)tau, $A\beta_{1-42}$ levels were immeasurably low, confirming a previous study describing post mortem CSF $A\beta_{1-42}$ levels.[24] Intra vitam $A\beta_{1-42}$ is an extracellular protein known for its tendency to form aggregates either in CSF or, as part of Alzheimer pathology, in plaques located in between neuronal cells. The extremely low $A\beta_{1-42}$ levels suggest that CSF $A\beta_{1-42}$ aggregates on a large scale after death, and that it is not recognized by the ELISA in its aggregated form. Another possible explanation for the extremely low $A\beta_{1-42}$ levels is that the $A\beta_{1-42}$ aggregates have precipitated in plaques in the brain, and are not present anymore in post mortem CSF.

In summary, CSF biomarkers seem to reflect neuropathology fairly well. Unfortunately, neuropathology does not always seem to correlate with the clinical presentation of signs and symptoms as demonstrated in previous neuropathological studies.[23,47,48] Thus, subjects with Braak stage VI have been described without dysfunction in daily activities as well as subjects with Braak stage II, that are clinically demented. This is a complicated issue, especially when physicians have to decide which subjects should be treated with anti-Alzheimer therapy that is directed against neuropathological abnormalities of AD. The next chapter combines CSF biomarkers with the MRI (or FDG-PET) scan hallmarks of AD, which may solve this issue.

3. Combination of CSF biomarkers and MRI for diagnosing Alzheimer's disease

Both CSF biomarkers and MRI are increasingly used to detect and characterise brain changes associated with AD. And although both MRI and CSF biomarkers have shown to be valuable markers of disease in MCI and AD,[49,50] the relation between these

markers has been less well studied, as well as the relation between these markers and cognitive function. Recently a cross-sectional relation was demonstrated between CSF tau levels and neuropsychological test performance in MCI patients.[51] Furthermore, in cross-sectional studies, CSF biomarkers have been reported not to be related to MRI measures of atrophy, suggesting that these markers reflect different aspects of Alzheimer type neuropathology.[52;53] The few studies that have reported both CSF biomarkers and MRI measures in a longitudinal design, have used relatively small sample sizes, and have shown conflicting results in terms of whether or not these markers are associated.[54-56]

In this thesis first, both CSF and MRI were combined to assess the predictive value in a large sample of MCI patients with substantial follow-up. Our data demonstrate that both CSF biomarkers and abnormal MTA-score are predictive of progression to dementia, mostly AD. The predictive value of CSF biomarkers was three times higher than the predictive value of the MTA-score, suggesting that CSF biomarkers may predict progression to dementia earlier in the course of the disease. Thus, the lower risk estimate for the MTA-score may reflect that an abnormal MTA-score predicts a swift progression to dementia, while the three times higher risk estimates of CSF biomarkers reflects their predictive value at a longer term. This corresponds with the presumed neuropathological course of AD: plaque and tangle formation, resulting in abnormal CSF biomarkers, precede neuronal loss. Neuronal loss results in atrophy that, especially when located in the medial temporal lobe, may precede measurable cognitive impairment.[57] The assumption that CSF biomarkers may predict progression to dementia earlier in the course of the disease is also confirmed by recent studies, with even higher risk estimates for developing AD in MCI patients.[58-60] Moreover, it was already discussed in chapter 1 of this discussion that abnormal CSF biomarkers may be predictive of cognitive decline in non-demented older adults, suggesting that CSF biomarkers can detect the underlying disease process even during the pre-clinical stage.[28-31]

In addition we found that, MCI patients with both abnormal CSF profile and an abnormal MTA-score, were at a fourfold higher risk to progress to AD compared to patients with either an abnormal CSF profile or an abnormal MTA-score. This may have important clinical implications, as it suggests that the ability to detect patients with MCI who are at risk for dementia, especially AD, will increase when CSF analysis is combined with measures of MTA. As such, next to structural neuroimaging, CSF analysis should be considered in the initial evaluation of memory clinic patients not yet meeting criteria of dementia.

Second, the relationship was assessed between baseline levels of CSF biomarkers and whole-brain atrophy rate in patients with AD, MCI, and controls and the association between longitudinal change of CSF biomarker levels, whole-brain atrophy rates, and change in cognitive function. Notwithstanding modest correlations of baseline CSF biomarker levels and whole-brain atrophy rate across groups, hardly any association within diagnostic groups was found. Whole-brain atrophy rate was associated with clinical progression, measured by change in MMSE score, but longitudinal changes in the CSF biomarker levels were not. Thus, MRI and CSF biomarkers appear to reflect different aspects of AD: whole-brain atrophy rate appears to be linked to the clinical progression of the disease, whereas CSF biomarkers seem to reflect disease state rather than rate of progression.

The reason that CSF biomarkers do not seem to reflect disease progression over time is not clear. Possibly, pathological CSF biomarker levels reflect a threshold phenomenon: as soon as histopathological changes in the brain occur, levels become pathological, and do not change over time. Since pathological changes of AD may occur as early as 20 to 30 years before clinical diagnosis, the MCI stage may be too late in the course of the disease to detect a transition from normal to pathological CSF biomarker levels. We included a sample of patients with subjective complaints, supposedly a clinical stage before MCI, but we were not able to demonstrate such a transition during the period under study. Moreover, cross-sectionally, one would expect a bi-modal distribution of CSF biomarker level. The continuous distribution that is in fact observed does not support the idea of a threshold phenomenon.

Post mortem studies have shown considerable overlap in the neuropathological features associated with AD, regardless of whether or not dementia was actually present during life.[47] This implies that other factors than senile plaques and neurofibrillary tangles must be involved in the development of the clinical syndrome of dementia. Indeed, it has been reported that brain volume by itself is a good predictor of dementia, independent of senile plaque and neurofibrillary tangle load.[47] Our results are in line with these neuropathological findings, since we found hardly any association of whole-brain atrophy rates and CSF biomarker levels. This could imply that brain volume loss in vivo, measured with MRI, and CSF biomarker levels, which are thought to represent senile plaque and neurofibrillary tangle load, reflect different aspects of AD. This is supported by the finding that longitudinal changes in CSF biomarker levels were not associated with change in MMSE, while whole-brain atrophy rates were. In clinical practice this may mean that for tracking the rate of progression of AD, whole-brain atrophy rates are more useful than CSF biomarkers; by contrast CSF biomarkers can be considered to be disease state markers, which may be more sensitive as diagnostic tools, possibly in earlier stages of AD.

Third and finally the newly proposed research criteria for AD were applied to our memory clinic population, combining memory dysfunction as core criterion with at least one supportive feature i.e. abnormal MTA score or abnormal CSF biomarkers.[61] Compared with the non demented group a good specificity of 96% (sensitivity 86%) was found. In the other dementias group, however, specificity was only 50%. When both supportive features, i.e. MTA score and CSF profile were abnormal, in addition to the core criterion of memory dysfunction the specificity increased to 78%. This confirms our study in MCI patients, where we also observed a higher risk for developing AD when both MTA score and CSF biomarkers were abnormal, compared to only one of these biomarkers being abnormal.

Thus, absence of MTA does not exclude AD and in the early stages especially young AD patients often lack prominent MTA.[62] In a study comparing young and old AD patients with age matched controls both old patients and old controls were found to have more MTA than young patients and controls, suggesting an additive effect of the factors age and diagnosis.[63] This is in contrast with our CSF biomarkers study, where a clear age effect was observed among controls, but both young and old AD patients had comparably abnormal biomarker levels. This suggests that especially in young patients, CSF biomarkers have more discriminative value than MRI markers.[53]

A possible limitation is the fact that MRI was part of the diagnostic work up of our memory clinic population, e.g. to exclude other neurological diseases like brain tumor.[64] Using MRI during the diagnostic process, may have slightly influenced our results, as the presence of MTA may unconsciously have influenced the physicians to a diagnosis of AD. We therefore cannot entirely exclude the possibility of circular reasoning.

We feel that, when there is clinical doubt about the type of dementia, two (instead of the presently proposed one) supportive feature should be abnormal in addition to the core criterion of memory impairment, especially in case of therapeutic trials with anti-Alzheimer therapies.

4. Future perspectives

General application of CSF biomarkers in clinical practice is hampered by the fact that the present studies all lack prolonged clinical follow up. As pathological changes in AD occur 20-30 years before the first signs and symptoms appear, a clinical follow up of at least 10-15 years would be ideal, to assess the predictive value of CSF biomarkers and their ability to reflect neuropathology. Unfortunately, at present the most extensive mean clinical follow-up time in MCI patients is 5-6 years. Note that this accounts for MCI patients, who are symptomatic already. A recent study showed some predictive

value of CSF biomarkers in non demented older subjects.[29] Although this finding is encouraging, the follow up time in this study was maximum eight years. This shows that future research is needed with extensive clinical follow up including cognitively normal subjects to assess the predictive value of CSF biomarkers and their usefulness for clinical practice in the diagnosis and therapy of AD.

Another lack is studies with neuropathological confirmation. So far only few studies assessed the diagnostic performance of CSF biomarkers compared to the neuropathological diagnosis consistently yielding sensitivities and specificities above 80%.[65-68] The last study, that included also autopsy material for a considerable number of controls, found a greater specificity and sensitivity for CSF biomarkers compared with the neuropathological diagnosis than compared with the clinical diagnosis. On the one hand, this confirms that CSF biomarkers are very well able to reflect neuropathological changes related to AD. On the other hand, AD pathology with plaques and tangles does not always seem to cause dementia or cognitive disorders, as demonstrated in previous neuropathological studies, which was also discussed in chapter 2 of the general discussion in this thesis.[23,47,69]

The fact that neuropathology does not always seem to correlate with the clinical presentation of signs and symptoms is a complicated issue. Especially when physicians have to decide which subjects should be treated with anti-Alzheimer therapy that is directed against neuropathological abnormalities of AD. The promising new PIB-PET technique, that visualizes amyloid plaques in the brains of AD and MCI patients, and also of asymptomatic subjects, will not be able to circumvent this problem. Future studies with therapy specifically directed against AD pathology should focus on AD patients that have several abnormal biomarkers (i.e. both on MRI, FDG-PET (or PIB-PET) and in CSF) in addition to clinical symptoms of cognitive impairment or dementia to optimize the chance of attacking plaques and tangles in the brains of these patients that actually cause cognitive dysfunction. In the far future, with vaccination therapy, it might be possible to apply primary prevention of developing AD, thereby expelling AD as a disease like small pox has been expelled, rendering all above mentioned biomarkers redundant.

Although post mortem CSF is not useful with the currently used AD biomarkers, it might be used for identification of new proteins as potential biomarkers of neurodegeneration.[43] Also intra vitam CSF may contain proteins, yet to be discovered, that could help in the diagnosis of incipient AD during life. Studies on intrathecal chemokine synthesis show that some chemokines may be elevated in the MCI stage of the disease and decrease with progression to AD while other chemokines are upregulated also in the late stages of the disease.[70,71] In addition, recently accomplished

proteomic studies, show a panel of proteins that is able to discriminate AD patients from controls and incipient AD in MCI patients.[72] Possibly these new proteins are more suitable for monitoring progression of disease and effect of therapy, since the currently used CSF biomarkers seem to be insensitive for progression of disease over time. Alternatively standardization of the presently used assays could be optimized, thereby reducing the variability and increasing the sensitivity to change over time.

Another possible source of biomarkers can be found in body fluids like plasma or urine. Plasma and serum or urine biomarkers proposed for AD are, just like CSF biomarkers, based on the pathofysiological processes such as amyloid plaque formation (Aβ, Aβ autoantibodies, platelet APP isoforms), inflammation (cytokines), oxidative stress (vitamin E, isoprostanes), lipid metabolism (apolipoprotein E, 24S-hydroxycholesterol), and vascular disease (homocysteine). Some studies in patients with Down syndrome, who are known to have neuropathological changes characteristic of AD, found plasma neopterin and $A\beta_{1-42}$ levels to be associated with AD.[73-75] Few studies found plasma $A\beta_{1-42}$ or inflammatory proteins to be elevated before the onset of cognitive decline in non-demented elderly or MCI patients,[76-78] and a study of white matter lesions on MRI found Aβ1-40 to be increased in AD and vascular dementia.[79] A recent study found 18 signalling proteins in plasma that discriminated AD from controls with 90% accuracy and identified MCI patients who progressed to AD 2-6 years later. Biological analysis of the 18 proteins pointed to systemic deregulation of haematopoiesis, immune responses, apoptosis and neuronal support in presymptomatic AD.[80] Studies on biomarkers in urine are even fewer, including small patient samples only.[79,81-83] In summary, most proteins or metabolites evaluated in plasma and serum or urine thus far are, at best, biological correlates of AD: levels are statistically different in AD versus controls in some cohorts, but they lack sensitivity or specificity for diagnosis or for tracking response to therapy. Approaches combining panels of existing biomarkers or other techniques like proteomics or urea based electrophoresis show promise for discovering biomarker profiles in plasma or urine that are characteristic of AD, yet distinct from non-demented patients or patients with other forms of dementia.[84,85]

Recommendations

1. In case of repeated spinal taps, determination of CSF $A\beta_{1-42}$ levels should be performed in the same assay.
2. Repeated assessment of CSF $A\beta_{1-42}$, total tau and ptau-181 levels is currently not useful in a clinical setting, as they are insensitive to disease progression.
3. When interpreting CSF $A\beta_{1-42}$, total tau and ptau-181 levels in clinical practice, it should be kept in mind, that elderly controls may have asymptomatic AD pathology, with corresponding pathological CSF levels, even in the absence of objective cognitive impairment.
4. Determination of CSF $A\beta_{1-42}$, total tau and ptau-181 levels in post mortem CSF is not useful for discrimination of neurodegenerative diseases.
5. Next to structural neuroimaging, CSF analysis should be considered in the initial evaluation of memory clinic patients.
6. When there is clinical doubt about the type of dementia, at least two biomarkers (MTA, CSF, FDG-PET) should be abnormal in addition to the core criterion of cognitive impairment, especially in case of therapeutic trials with anti-Alzheimer therapies.

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Chapter 7

Nederlandse samenvatting

Curriculum Vitae

List of publications

Dankwoord

Nederlandse samenvatting

Het doel van het onderzoek dat in dit proefschrift is beschreven, was om inzicht te krijgen in de longitudinale aspecten van liquorbiomarkers en de post mortem veranderingen van deze biomarkers. Verder werd vergeleken in hoeverre liquorbiomarkers danwel MRI (beginnende) ziekte van Alzheimer (ZvA) konden detecteren bij geheugenpolikliniekpatienten.

Ten eerste vonden wij een hogere variabiliteit tussen baseline en follow-up $A\beta_{1-42}$ concentraties wanneer ze werden bepaald in verschillende assays ten opzichte van bepaling in dezelfde assay. Dus, in het geval van meer dan één lumbaal punctie bij eenzelfde patient, adviseren wij de $A\beta_{1-42}$ concentraties in dezelfde assay te bepalen. Vervolgens beschreven wij dat het natuurlijke longitudinale beloop van $A\beta_{1-42}$, tau en ptau-181 concentraties in de liquor vergelijkbaar was tussen patienten met subjectieve geheugenklachten, mild cognitive impairment (MCI) en ZvA. De cross-sectionele verschillen tussen de groepen overschreden echter ruimschoots de longitudinale veranderingen. Herhaalde bepaling van deze liquorbiomarkers in een klinische setting achten wij dan ook niet zinvol, omdat ze ongevoelig zijn voor ziekte progressie.

Ten tweede vonden wij, dat jonge en oude patienten met ZvA vergelijkbare biomarker concentraties in de liquor hadden, terwijl oudere controles een lagere $A\beta_{1-42}$ en hogere tau en ptau-181 conentratie hadden in vergelijking met jongere controles. Dit zou kunnen suggereren dat er sprake is van preklinische Alzheimer pathologie bij oudere controles. Daarmee dient rekening te worden gehouden bij het interpreteren van liquorbiomarkerconcentraties in de klinische praktijk. Verder wordt mogelijk een verschil in Alzheimer pathologie gesuggereerd tussen jonge en oude patienten met ZvA, want ookal hebben jonge en oude patienten absoluut gezien dezelfde liquorbiomarkerconcentraties, relatief gezien, ten opzichte van hun controle groep, hebben jonge patienten met ZvA pathologischer liquorbiomarkerconcentraties.

Ten derde concludeerden wij dat bepaling van $A\beta_{1-42}$, tau en ptau-181 in post mortem liquor niet zinvol is, omdat (p)tau concentraties extreem hoog bleken en $A\beta_{1-42}$ concentraties onmeetbaar laag, zonder dat er verschil was in concentratie tussen patienten met ZvA of Lewy body ziekte en controles.

Ten vierde combineerden wij liquor en MRI en vonden we nauwelijks enige associatie binnen de diagnostische groepen, voor wat betreft liquorbiomarkerconcentraties en toename van hersenatrofie bij controles, MCI en ZvA patienten. Wel waren er bescheiden correlaties tussen baseline liquorconcentraties en toename van hersenatrofie. Toename van hersenatrofie was geassocieerd met klinische progressie, gemeten met de MMSE-score, maar longitudinale veranderingen van liquorbiomarkers niet. Verder toonden

wij aan dat afwijkende $A\beta_{1-42}$ en tau concentraties en een afwijkende MTA-score geassocieerd zijn met een hoger risico op ontwikkelen van ZvA bij MCI patienten. De voorspellende waardes van liquorbiomarkers waren hoger dan van de MTA-score. MCI patienten met zowel abnormale liquormarkerconcentraties als een abnormale MTA-score hadden nog meer risico op het ontwikkelen van ZvA. Dit is in overeenstemming met wat we vonden bij de toepassing van de nieuwe onderzoekscriteria voor ZvA: de combinatie van zowel abnormaal liquorbiomarker profiel als abnormale MTA score bij een afwijkende geheugentest leverde een specificiteit op van 100% (sensitiviteit 43%), terwijl een abnormale geheugentest met ofwel abnormale MTA score ofwel abnormaal CSF profiel een specificiteit opleverde van 96% (sensitiviteit 86%). Wij concluderen dan ook dat MRI en liquorbiomarkers verschillende aspecten van ZvA lijken weer te geven: atrofie bepalingen op MRI lijken te zijn gerelateerd aan klinische progressie van de ziekte, terwijl liquorbiomarkers eerder de aan of afwezigheid van de ziekte weergeven dan de mate van progressie.

Curriculum Vitae

Femke Bouwman was born the 17th of July 1971 in Haarlem. In 1989 she graduated from Beekvliet (Ongedeeld Gymnasium) in Sint Michielsgestel. As she was not admitted to Medical School until 1991 because of the lottery system, she completed Schoevers medical and managing secretary and worked as a secretary in the Liduina Hospital in Boxtel. From 1991 till 1998 she studied medicine at the Erasmus University in Rotterdam. During her study she worked as a volunteer in Medical Hospital Apam (Ghana, West Africa) in 1993 and performed research in HIV associated dementia at the Hopkins University (Baltimore, USA) in 1995 and 1996. This resulted in her first publication in an internationally peer reviewed magazine (Neurology) and a young investigators award at the International Conference on AIDS at Vancouver in 1996. In 1998 she worked as a physician at the department of Neurology of the Academic Medical Centre in Amsterdam and in 1999 she started her specialist registrar neurology training at the Medical Centre Haaglanden (location Westeinde) in The Hague with Dr. J. Th. Tans and Dr Ch. Vecht as tutors. During her training as registrar she was a board member of the Dutch Association for Neurology registrars and junior member of the European Board of Neurology, representing all neurology registrars in Europe. In 2004 she began her research project at the Alzheimer Centre, Department of Neurology at the Vrije Universiteit Medical Center in Amsterdam, supervised by Prof. Dr. P. Scheltens and Prof. Dr. M.A. Blankenstein, which resulted in this thesis. During her research project, she participated in outpatient care both at the Alzheimer Centre and at the general Neurology department. Since January 2008 she is working as a neurologist at the memory clinic of the Catharina Hospital in Eindhoven where she will accede to the partnership neurology in July 2008.

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